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BIOMEDICAL ENGINEERING AND INSTRUMENTATION BRANCH

DIVISION OF RESEARCH SERVICES

NATIONAL INSTITUTES OF HEALTH

ANNUAL REPORT FY 1986

Dr. Murray Eden, Chief

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BIOMEDICAL ENGINEERING AND INSTRUMENTATION BRANCH

DIVISION OF RESEARCH SERVICES

NATIONAL INSTITUTES OF HEALTH

ANNUAL REPORT FY 1986

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RS 10001-18 BEI

PERIOD COVERED: October 1, 1985 to September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.): Pharmacokinetics

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

R.L. Dedrick	Chief, CHES	BEIB, DRS
Others:		
F. Farris	North Dakota State University	
R. J. Lutz	Chemical Engineer	BEIB, DRS
F. King	North Carolina A&T Univ.	
P. F. Morrison	Physical Scientist	BEIB, DRS

COOPERATING UNITS (if any): CPB, NCI (J.M. Collins); DR-CC (J.L. Doppman); NTP-NIEHS (H.B. Matthews); SNB-NINCDS (E.H. Oldfield); University of Maryland (M.Egorin).

LAB/BRANCH: Biomedical Engineering and Instrumentation

SECTION: Chemical Engineering Section

INSTITUTE AND LOCATION: DRS, National Institutes of Health, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects
  - ☐ (a1) Minors
  - ☐ (a2) Interviews
- ☐ (b) Human tissues
- ☒ (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Pharmacokinetic models are developed for the distribution and disposition of drugs, environmental contaminants, and endogenous metabolites in animals and man. They provide a plausible set of equations that can be used to extrapolate data from animals to man and thereby improve chemotherapy and risk assessment. Emphasis has been placed on regional drug administration, and this has led to the development of spatially distributed models of drug transport in tissue. These analyses have provided considerable insight into the penetration depths of drugs administered intraperitoneally or by infusion into the brain. Pharmacokinetic theory which was developed for intra-arterial drug administration combined with extracorporeal treatment of venous drainage has been further validated in the clinic. Preliminary results have shown the feasibility of documenting appropriate vascular drainage with indocyanine green. Interspecies scaling of regional drug administration has been explored, and a clear allometric basis has been elaborated.

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OBJECTIVES: Improve and extend mathematical models for the distribution and disposition of drugs, environmental contaminants, and endogenous metabolites in animals and man to:

(1) Account for species differences in drug distribution.

(2) Provide a rational basis for extrapolating toxicity from animals to man.

(3) In conjunction with pharmacodynamics, provide a basis for optimizing cancer chemotherapy.

(4) Enable rational transfer of in vitro thermodynamic and kinetic data to in vivo cases.

(5) Predict effective dose schedules of anticancer drugs in individual patients with particular emphasis on regional drug delivery.

METHODS EMPLOYED: Mathematical models are developed from physicochemical, physiological, and anatomical information and the principles of chemical reaction engineering. Resulting ordinary or partial differential equations are solved analytically or numerically and compared with experimental data. Uncertainties are clarified by additional experiments and model modification.

(1) The pharmacokinetic advantage of intra-arterial drug administration can be increased if blood from the infused region is passed through an extracorporeal device which removes drug before the blood is returned to the body. Three patients with malignant gliomas received infusions of DDP into the internal carotid artery at a dose of 200 mg/m^2 infused for 1 hour while blood from the ipsilateral jugular bulb was dialyzed through two hollow fiber hemodialyzers in series. Plasma samples were obtained at frequent intervals from the dialyzer inflow, dialyzer outflow and right atrium for measurement of total and filterable platinum. In addition, the dialysate outflow was assayed to confirm the material balance. The reduction in systemic dose could be calculated independently from the amount of platinum removed from the plasma and the amount of platinum appearing in the dialysate. Hemodialysis reduced systemic exposure to values comparable to or less than those observed in studies using a more conventional dose of 100 mg/m^2.

(2) Calculation of the pharmacokinetic advantage of regional drug administration requires knowledge of the relevant intercompartment transport parameter. In a lumped model this is the blood (or plasma) flow rate for intra-arterial drug infusion or the permeability-area product for intraperitoneal or intrathecal administration. It is suggested that the perfusion of many tissues and the intrinsic permeability of the peritoneal surface and the brain-cerebrospinal fluid interface are similar among mammals. This provides a clear allometric basis for interspecies scaling based on organ size or surface areas. Intra-arterial or intrathecal treatment of the brain or meninges is a particularly interesting problem because of the relatively large brain of humans and because increased folding results in a cortical surface area that is almost proportional to brain size. Major unresolved issues remain concerning the distributed character of the processes such as streaming of drug infused into an artery and nonuniform mixing of cerebrospinal fluid.

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SIGNIFICANCE: Drugs and other chemicals are tested for effect in animals, with the aim of extrapolating results to man. At issue are both the risk associated with environmental contaminants and optimization of therapy.

PROPOSED COURSE: Continued pharmacokinetic modeling with consideration of pharmacodynamic and cytokinetic events and drug interactions. Continued support of regional procedures and other measures to overcome drug resistance. Research designed to investigate distribution and metabolism of environmental contaminants and on methods for incorporating pharmacokinetics in models of risk assessment. Investigation of use in in vitro assays of chemical metabolism in conjunction with pharmacokinetic models for quantitative prediction of metabolism in vivo. Extension of distributed models for description of drug movement through tissue. Study of the transport of macromolecules and lymphocytes, particularly Lymphokine Activated Killer (LAK) cells and Interleukin-2.

PUBLICATIONS

Dedrick, R.L., Flessner, M.F., Schultz, J.S.,: Transport between the peritoneal cavity and the vascular space,. Proc. Ann. Conf. Eng. Med Biol. 27: 46.1, 1985.

Dedrick, R.L.: Theoretical and experimental bases of intraperitoneal chemotherapy Sem. Oncol. 12: 1-6, 1985.

Dedrick, R.L., Flessner, M.F., Collins, J.M., Schuultz, J.S.: A distributed model of peritoneal transport, In Frontiers in Peritoneal Dialysis, J.F. Maher and J.F. Winchester (Eds.), Field, Rich and Assoc., New York, 1986, pp 41-46.

Flessner, M.F., Dedrick, R.L., Fenstermacher, J.D., Blasberg, R.G., Sieber, S.M.: Peritoneal absorption of macromolecules In frontiers in peritoneal dialysis, J.F. Maher and J.F. Winchester (Eds.), Field, Rich and Assoc., New York, 1986, pp 41-46.

Oldfield, E.H., Dedrick, R.L., Yeager, R.L., Clark, W.C., DeVroom, H.L., Chatterji, D.C. and Doppman, J.L., Reduced systemic drug exposure by combining intra-arterial chemotherapy with hemoperfusion of regional venous drainage, J. Neurosurg. 63: 726-732 1985.

Morrison, P.F., Dedrick, R.L.: Transport of cisplatin in rat brain following microinfusion: an analysis, J. Pharm. Sci. 75: 120-128 1986.

Doppman, J.L., Dedrick, R.L., Shook, D.R., Lutz, R.J., Goldstein, S.R., Blacklock, J.B., Boretos, J.W., Paul, R.H., Austin, H.A., Bowman, R.L., Oldfield, E.H., Glioblastoma: Catheter techniques for isolated chemotherapy perfusion, Radiology 159: 477-484 1986.

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King, F.G., Dedrick, R.L., Farris, F.F.: Physiological pharmacokinetic modeling of cis-dichlorodiammineplatinum (II) in several species, J. Pharmacokin. Biopharm. 14: 131-155 1986.

Dedrick, R.L.: Application of model systems in pharmacokinetics. In Silvers, A. and Newell, G.W. (Eds.): New Directions on the Extrapolation of Health Risks from Animals to Man. vol. 2 Toxicokinetics. Electric Power Research Institute, Palo Alto, CA 1986. pp. 6:1 - 6:20.

Collins, J.M., Zaharko, D.S., Dedrick, R.L., Chabner, B.A.: Potential roles for preclinical pharmacology in phase I clinical trials, Cancer Treatment Rep. 70: 73-80 1986.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RS 10002-21 BEI

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Implant Device Development

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

John W. Boretos, Physical Scientist, BEIB, DRS

John Doppman, M.D.	Radiologist	CR, CC, NIH
Edward Oldfield, M.D.	Neurosurgeon	NINCDS, NIH
F.T. Hambrecht, M.D.	Health Science Adm.	NINCDS, NIH
Richard Clark, M.D.	Heart Surgeon	NHLBI, NIH
Donald Wright, M.D.	Neurosurgeon	NINCDS, NIH

COOPERATING UNITS (if any)
CR, CC, NIH; NS, NINCDS, NIH;
FNP, NINCDS, NIH; S, NHLBI, NIH;
BEIB, DRS, NIH.

LAB/BRANCH
Biomedical Engineering and Instrumentation

SECTION
Chemical Engineering

INSTITUTE AND LOCATION
DRS, NIH Bethesda, MD. 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	0.7	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this project is to elucidate the interaction of biomaterials used for specific implants with the physiological environment and to explore specially prepared biomaterials and design features with respect to their suitability and performance in a variety of contexts. Polymers, metals and ceramics are important for use in catheters, heart assist pumps, artery cuffs, electrode insulation and similar implant applications. Variations in these materials as well as physically induced stress and environmentally accelerated degradation can severely reduce their effectiveness for long-term use as a surgical device. Previous studies undertaken by this project have shown a relationship between the molecular chain structure and resistance to hydrolysis. Recent evidence suggests that physical forces such as stress induced during fabrication can promote a form of stress corrosion. In vitro test data and SEM photomicrographs of surgical explants of various polyurethane classes show that premature failure is often the result of a combination of forces acting on the polymer at stress risers. Polymer systems and composites capable of timed dissolution offer significant advantages in the development of devices that allow natural tissues to take over as healing progresses. Tissue ingrowth into porous materials provides greater stability for specific implants. A strong correlation exists between these in vitro and in vivo observations over short and long term periods of study.

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OBJECTIVES: Elucidate the interaction of polymers, metals, and ceramics used for specific implants with the physiological environment; explore specially prepared polymers, their properties and design features with the physiological environment in a variety of contexts.

METHODS EMPLOYED: Basic composition of biomaterials is carefully controlled, and modifications are employed to enhance acceptability by the living system. After removal, implants are examined for lipid absorption, protein and/or calcium deposition, changes of surface-free energy and alteration of physical properties. Observation techniques include SEM, infrared spectroscopy, contact angle measurements, energy dispersive x-ray analysis, and atomic absorption spectroscopy. Electronic implants are examined periodically in vivo for changes in threshold levels, corrosion, and tissue activity. In vitro studies of the aforementioned are designed to accelerate fatigue testing and methods for improvement of components undergoing stress.

MAJOR FINDINGS: Polyurethanes are a complex and varied class of polymers whose full potential for medical use has not been achieved. Characterization of its behavior under conditions of use is necessary to project its safety and efficiency as a surgical implant. Variations in the basic chemical structure of these polymers as well as physically induced stress can severely reduce their usefulness. Previous studies undertaken by this project have shown a direct relationship between the molecular structure of the polymeric chain and its ability to resist hydrolysis.

SIGNIFICANCE: Physiologically compatible polymers with properties specifically tailored for surgical implants, indwelling catheters, and subcutaneous uses are needed.

PROPOSED COURSE: Extend experimental studies to characterize further the surface and bulk properties of biomaterials and, more specifically, to determine their interactions with blood and subcutaneous tissue to facilitate development of better surgical implants.

PUBLICATIONS

Boretos, J.W., Girton, M.E., and Doppman, J.L.: Polyurethane radiopaque marker for indwelling devices. J. Biomed. Mater. Res. 20: 273-275 1986.

Boretos, J.W.: Ceramics in clinical care. The Bulletin of The American Ceramic Society. August 1985 pp 1098-1100.

Boretos, J.W.: Sullivan, J.V., Fitze, P.E., Girton. M.E. and Doppman, J.L.: Jet-Directed Catheter for Interventional Radiology. Annals Biomed. Engr. 14, 323-338, 1986.

Doppman, J.L., Dedrick, R.L., Shook, D.R., Lutz, R.J., Goldstein, S.R., Blacklock, J.B., Boretos, J.W., Paul, R.H., Austin, H.A., Bowman, R.L. and Oldfield, E.H.: Glioblastoma: Catheter Techniques for Isolated Chemotherapy Perfusion Radiology. 159 (2) 477-483, 1986.

Lutz, R.J., Dedrick, R.L., Boretos, J.W., Oldfield, E.H., Blacklock, J.B. and Doppman, J.L.: Mixing Studies during Intracarotid Artery Infusions in an in vitro Model. J. Neurosurg. 64, 277-283, 1986.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RS 10015-11 BEI

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Development of Everting (Toposcopic) Catheter for Clinical Vascular Use

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: D.R. Shook, Biomedical Engineer,	BEIB, DRS
J.L. Doppman, Chief,	DRD, CC
E.H. Oldfield, Chief (Acting)	SNB, NINCDS
D.L. Miller, Radiologist	DRS, CC
H.R. Keisar, Clinical Director	DIR, NHLBI

COOPERATING UNITS (if any)
Diagnostic Radiology, CC (J.L. Doppman, D. Miller);
Surgical Neurology, NINCDS (E.H. Oldfield)

LAB/BRANCH
Biomedical Engineering and Instrumentation Branch

SECTION
Mechanical Engineering Section,

INSTITUTE AND LOCATION
DRS, National Institutes of Health, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The everting catheter has been shown to be a reliable clinical means to catheterize long, small diameter, and highly tortuous blood vessels, inaccessible by previous techniques. An everting element evaginates from the tip of a conventional catheter. This extremely flexible polyurethane element has been fabricated in 3, 4 and 5 French sizes mated with 5, 6 and 7 French catheters, respectively, and is capable of eversion lengths in excess of 40 cm.

The catheter has been applied clinically for the local delivery of chemotherapy to brain tumors and of embolizing agents in the liver. In the former, treatment is provided by positioning the conventional catheter in the internal carotid artery from a femoral entry; everting the element through the carotid sinus, beyond the ophthalmic artery to avert retinal toxicity; and perfusing the tumor through the middle and/or anterior cerebral arteries. Note that a conventional catheter cannot safely negotiate the tortuosity of the carotid sinus. In the latter application, embolizing agents are delivered intra-arterially to block the perfusion of hepatic tumors. The sinuousity of the vessel precludes access by conventional techniques.

The catheter has been extensively used clinically in its present prototype form, and production techniques for its fabrication have been transferred to private industry to provide for a ready source of catheters for future studies.

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OBJECTIVES: Develop techniques for selective toposcopic catheterization of small, tortuous blood vessels and optimize apparatus for varied diagnostic and therapeutic applications.

METHODS: Catheters for vascular use are constructed of custom extruded polyurethane tubing. Fabrication techniques for prototypes are manual and individually designed. These methods allow for optimization of the catheter for specific applications. Equipment for polymer processing, materials testing, bonding, coating, and heat treating is readily available. Production techniques are, in general, not applied; initial designs are developed with ultimate mass production of the device always considered.

MAJOR FINDINGS: Eversion, infusion, and retraction of the catheter have occurred without incident in over forty clinical chemotherapeutic treatments. Implementation of existing techniques to direct catheter eversion is required in some difficult anatomical situations. Continued follow-up of brain tumor patients has suggested that treatments at low infusion rates may be hampered by inadequate infusate/blood mixing. Recent literature reports indicate that this problem is, to a great extent, independent of the catheter and more a consequence of the minute flow rates involved (1 ml/min). Therefore, independent studies have been initiated to fully characterize the problem and to evaluate the effectiveness of pulsed intra-arterial infusion as a clinical solution.

SIGNIFICANCE: By atraumatically negotiating small, highly tortuous blood vessels, the catheter reaches heretofore inaccessible regions for both diagnostic and therapeutic application. Thus, routine fluoroscopic examinations are readily augmented by selective treatment modalities for tumors, aneurysms, arterio-venous malformations, and other lesions which previously required major surgical interventions.

PROPOSED COURSE: Optimize catheter system for diverse clinical applications. Transfer production technology to private industry for ready source of catheters. Investigate significance of infusate/blood mixing efficiency at tip of catheter. Develop wire guiding, remote steering and arterial dilatation methods. Develop related devices and explore additional uses for the catheter.

PUBLICATIONS

Shook, D.R., Doppman, J.L., Cattau, E.L., Jr., Goldstein, S.R.: Everting (Toposcopic) catheter for broad clinical application. J. Biomechanical Engineering, 108: 168-174, 1986.

Related Project Number:

Z01-RS-10001-18 BEIB
Z01-RS-10193-03 BEIB (GI use)
Z01-RS-10194-03 BEIB (Polymer Processing)
Z01-RS-10206-01 BEIB (Pulsed Intra-arterial Infusions)
Z01-RS-10226-01 BEIB (Fallopian Tube Use)

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RS 10034-09 BEI

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Three-Dimensional Histological Reconstruction

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

S.B. Leighton, Mechanical Engineer, BEIB, DRS
A.M. Kuzirian, Neuroanatomist, LB, NINCDS

COOPERATING UNITS *(if any)*

LB NINCDS

LAB/BRANCH
Biomedical Engineering and Instrumentation

SECTION
Mechanical Engineering Section

INSTITUTE AND LOCATION
National Institutes of Health, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.2	.2	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A semi-automatic system for acquiring three-dimensional structural information about histological material is being developed. The system should have significant speed and reliability advantages over present techniques using serial sections, although resolution may be limited. In brief, an embedded tissue block will be fixed relative to a scanning electron microscope imaging system, the surface of the block will be imaged and the image stored, and successive slices will be removed by a built-in microtome. Handling and registration of thin sections will thus be eliminated. Human and computer pattern recognition will transform the resulting set of images into a three-dimensional reconstruction. Oxygen plasma etching has been found to give sufficient topographic relief that the resolution of the images is now limited by the SEM and not by the preparation technique. The images of Hermissenda Crassicornis obtained by this technique correlate well with TEM images of the same tissue, indicating that the lack of artifact is adequate for the contemplated studies.

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OBJECTIVES: (1) To facilitate making schematic diagrams of neural networks. (2) To facilitate developmental studies of small organs and organisms. (3) To do three-dimensional reconstruction of biological structures.

METHODS EMPLOYED: A miniature microtome has been built to function within the vacuum chamber of a scanning electron microscope. The microtome has cut sections as thin as 0.5 microns. Epoxy has been tried as a embedding medium, and oxygen plasma as an etching means.

MAJOR FINDINGS: The microtome knife and specimen feed mechanisms have been rebuilt for all electronic operation to facilitate interfacing with a computer and with a new SEM, and to remove the risk of fluid leaks into the SEM. The new specimen drive is capable of 5000 Angstrom section thickness. The miniature gold gun and small etching antenna have been found inadequate for extended use, but new designs have been devised for both. Total time per image (cut, etch, coat, view) is expected to be less than 7 minutes. The system has been integrated with a new SEM in LB, NINCDS.

SIGNIFICANCE: Neuroanatomists may be able to trace significant neural nets with sufficient ease to allow a statistically significant number of samples. Other biological studies may be materially aided.

PROPOSED COURSE: The new designs for the gold gun and etching antenna will be built and tested. Parameters such as etching time and intensity will be optimized. We will attempt more routine use of the instrument, and will try an actual reconstruction of a small neural network in Hermissenda Crassicornis. X-ray image analysis will also be tried. Computer control will be planned.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RS 10039-09 BEI

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Biophysical Instrumentation and Methodology

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Marc S. Lewis, Research Chemist, BEIB/DRS

Other: Thomas R. Clem, Electronics Engineer BEIB/DRS

COOPERATING UNITS (if any)

LAB/BRANCH
Biomedical Engineering and Instrumentation

SECTION
Analytical Methods

INSTITUTE AND LOCATION
DRS, National Institutes of Health, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The project is designed to develop new instrumentation and methodology or improve existing instrumentation and methodology for characterization of biological macromolecules and for studying their interactions. Analytical ultracentrifugation, the techniques ancillary to it, and methods of data analysis using mathematical modeling appropriate for these techniques are the major areas of interest.

The major emphasis in this project has been given to the development of new applications of the techniques of mathematical modeling to problems in ultracentrifugal analysis. The two areas this project has been concerned with have been an extension of the method of implicit constraints to a wider range of studies of macromolecular interactions and a new approach toward obtaining molecular weight distributions in solutions of macromolecular biopolymers. MLAB, operating on the DEC-10 computer, has been used for the mathematical modeling. The applications of these studies are described in the annual report entitled Physical Chemistry of Biological Macromolecules.

No new work on data acquisition has been carried out except for the continuation of a study on the possibility of development of new optical data acquisition systems for the conversion of current preparative ultracentrifuges into analytical ultracentrifuges with superior capabilities as analytical instruments at a fraction of the cost of the current instruments. New preliminary designs for such systems have been developed.

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OBJECTIVES: To develop data acquisition systems for analytical ultracentrifuges and for ancillary equipment such as plate or film readers and densimeters, to develop appropriate software to use with the acquisition systems, to develop new instrumentation for the conversion of preparative ultracentrifuges into analytical ultracentrifuges and to study means of utilizing MLAB more effectively for the analysis of ultracentrifuge data by mathematical modeling techniques.

METHODS EMPLOYED: Mathematical modeling studies have involved the use of computer simulations as well as the analysis of experimental data. The objective of the method of implicit constraints is to write a mathematical model to be used for data analysis in such form that the minimum number of parameters possible will be used and that all parameter values will be constrained to real and physically meaningful values. A simple example of this is to fit for the logarithms of equilibrium constants and concentrations, thus limiting the equilibrium constants and the concentrations to positive values. Of greater complexity is to take into account the initial concentrations of the interacting species and utilize conservation of mass in the ultracentrifuge cell. The problem of obtaining molecular weight distributions (concentration as a function of molecular weight) from concentration as a function of radius in the ultracentrifugal cell is a mathematically ill-conditioned problem and attempts at solutions have met with little success in the past. The approach taken here has been to seek an approximate solution by postulating a possible distribution, then obtaining the mean, standard deviation and skewness of that distribution as fitting parameters, and then estimating the quality of the approximation by the root-mean-square error and the plot of the deviations from the fitting line. The prospect that the manufacturer's support for the current analytical ultracentrifuge will become more limited in future years has stimulated interest in the development of new optical systems with data acquisition systems for converting the present excellent preparative ultracentrifuges into analytical instruments. Preliminary designs for such systems have been developed.

MAJOR FINDINGS: The method of implicit constraints has been applied with considerable success to studies of interacting systems involving 1:1 and 1:4 stoichiometries. In addition, models for application to other stiochiometries have been developed, and it is expected that, in view of the sucess of the method and its significant advantages for the analysis of interacting systems, a manuscript describing it will be submitted in the next year. Simulation studies on the analysis of molecular weight distributions have been rather successful for unimodal distributions of thermodynamically ideal biopolymers. However, the analysis of biomodal distributions has not been at all successful, and while the problem appears to lie in developing a better method of estimating initial parameter values and appropriate constraints, analysis of such distributions may not be possible by this method. The analysis of thermodynamically non-ideal systems has not been attempted as yet. Preliminary studies indicate that it appears possible to develop very compact optical systems which will fit entirely within the vacuum chamber of a preparative ultracentrifuge, be easily removable to permit the normal preparative use of the instrument, and yet be capable of offering better precision than the current analytical ultracentrifuge.

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SIGNIFICANCE: Improved methods of data analysis by mathematical modeling contribute significantly to the quality and the time and cost effectiveness of research involving analytical ultracentrifugation. The development of a new analytical ultracentrifuge will greatly facilitate such studies and will free the investigator from the constraints of obsolescent instrumentation.

PROPOSED COURSE: Continue the developments outlined above.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RS 10043-09 BEI

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Fiber Optic Probes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

John I. Peterson, Chemist, BEIB/DRS
Einar Stefansson, EICB

COOPERATING UNITS (if any)

RVR

LAB/BRANCH
Biomedical Engineering and Instrumentation

SECTION
Chemical Engineering Section

INSTITUTE AND LOCATION
DRS, National Institutes of Health, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.8	.8	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A suitable device is needed for the direct measurement of oxygen partial pressure (PO_2) in blood and tissue for both clinical and research applications. Methods currently available for measuring PO2 lack convenience, reliability, speed, and relevance to many situations of interest. Efforts to develop electrical sensors have not been successful. It is desirable to have a very small PO2 sensor which can be inserted into a blood vessel or tissue with little disturbance, and which will provide instantaneous and current PO2 monitoring for either short or extended periods of time. A fiber optic sensor is ideal for this application, with the advantage, for physiological use, of very small size and flexibility, safety, and low cost. A PO2 sensor has been developed, based upon the principle of fluorescence quenching by oxygen. The feasibility of the sensor and its satisfactory performance has been demonstrated in in vitro and in vivo tests, in work in preceding years. In the previous year, the development of the sensor as a needle probe was accomplished, and problems of storage life and stability were resolved.

During the past year, several problems relating to development of a usable instrument and probe were solved, and the system was used for experiments to measure PO2 in the eyes of dogs. This was done in comparison with polarographic electrode measurements of PO2. The experiments were done to evaluate the suitability of the sensor for studies of diabetic retinopathy.

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OBJECTIVES: Develop an oxygen sensor for implantation in the blood stream and tissues, to be used in studies of oxygen transport.

METHODS EMPLOYED: A fiber optic measurement of dye-indicator response to oxygen by fluorescence quenching.

SIGNIFICANCE: PO2 measurements are fundamental to the understanding and control of oxygen transport in research and clinical investigations. Measurements on withdrawn blood samples lack convenience, reliability, and relevance to many situations of interest. Indirect estimation of PO2 using spectrophotometric measurements of hemoglobin oxygenation and the concentration-pressure transfer function (blood oxygen saturation curve) is subject to many uncertain variables. Development of a fiber optic PO2 probe represents a significant advance in the ability to directly and continuously measure blood and tissue oxygen. A satisfactory electrode for general use has never been developed, and the fiber optic approach offers some distinct advantages in small size, flexibility, and safety.

MAJOR FINDINGS

1. Further work was done to perfect the construction of a needle probe for eye measurements.

2. Major changes in the instrument and probe design were made to correct a problem with baseline drift. This problem has prevented the application of the probe to measurements lasting several hours.

3. The system was used for experiments to measure PO2 in the eyes of dogs. This was done in comparison with polarographic electrode measurements of PO2. The experiments were done to evaluate the suitability of the sensor for studies of diabetic retinopathy.

4. A publication of the experimental work is in preparation.

PROPOSED COURSE

1. The immediate effort is to construct an instrument based on previous design improvements for use in continuing eye experiments with human subjects. This will necessitate additional modifications to allow the optical sensor to work in high light intensity.

2. Additional work which is planned is the use of the instrument for other projects, and the development of instrumentation and probes to allow measurement of pH and PCO2, along with PO2.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RS 10096-06 BEI

PERIOD COVERED: October 1, 1985 to September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Light Scattering Method for Evaluation of Platelets

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

R. F. Bonner Physicist BEIB DRS		
T.R. Clem	Elect. Engineer	BEIB DRS
S.B. Leighton	Mech. Engineer	BEIB DRS
J. Fratatoni	Dir. Blood Bank Products Branch	DBBP,CDB, FDA
B. Poindexter	Biologist	BBPB, DBBP, CDB, FDA
P. Mintz	Director	Blood Bank, U. Virginia

COOPERATING UNITS (if any)
Center for Drugs and Biologics, FDA
Blood Bank, U. Virginia

LAB/BRANCH
Biomedical Engineering and Instrumentation Branch

SECTION
Electrical and Electronic Engineering

INSTITUTE AND LOCATION
DRS, National Institutes of Health, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.4	0.4	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In the preceding years of this project we have developed a unique instrument to noninvasively assess the concentration and morphology of transfusion platelets in a rapid clinically acceptable procedure. The basis of this measurement is low-angle multiple scattering of light and the changes in this signal associated with orientation of the subset of the platelet population that are discoid. The instrument, as developed, is automated under microprocessor control and calculates the clinically important values of concentration and percentage that are discoid of the platelets in transfusion bag within a 3-minute period. These highly accurate measurements are being used in a variety of research studies of the effects of various storage conditions (e.g., temperature transients, bag wall material, addition of pharmacological agents) on the condition of stored platelet concentrates.

The instrument was originally designed to play a significant role in improving the quality of platelet transfusions, by routine and accurate noninvasive assessment of platelet transfusions, by routine and accurate noninvasive assessment of platelet quality (as represented by the degree or normal platelet morphology). To this purpose ongoing studies at the Univ. of Va. Medical School Blood Bank are directed at its clinical utility and determining the degree of correlation of the noninvasive measurement with clinical efficacy of the platelet transfusion.

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OBJECTIVES: The primary task of development of the method and automated instrument have been previously accomplished. The current objective is to facilitate ongoing clinical research evaluation of the quality of platelet transfusion and to develop optimal methods for the routine use of this technology in the practive of blood banking and transfusion medicine.

METHODS EMPLOYED: Human studies include the evaluation of apharesis platelet products at preparation and periodically during storage prior to transfusion in patients and the independent evaluation of platelet viability (survival) by radio-labelling platelets from normal volunteers prior to autologous transfusion. In patients being transfused platelet counts before and after transfusion (24 hours) provide a measurement to be correlated with the noninvasive optical measurements.

In vitro studies utilize the accurate and sensitive noninvasive measurements to evaluate changes in platelet morphology on exposure to cold and susequent rewarming, on rewarming to body temperature after room temperature storage, and on the effect of bag storage conditions (wall gas permeability, platelet and white blood cell counts, pH, etc.).

MAJOR FINDINGS: 1) The ease of clinical use and reproducibility of measurements has been established. 2) The valiability of product using state-of-the -art apharesis technology has been documented as well as indications of possible improvements in blood banking methodolgy. 3) The accuracy of the noninvasive "optical" platelet counts as compared with existing invasive measurement technology. 4) The detection and quantitation of reversible as well as irreversible changes in platelet morphology under normal conditions of platelet storage.

SIGNIFICANCE: This methodology and instrument has already proven to be a useful tool in transfusion research. Its suitability for routine blood banking use has been demonstrated. The future impact as the routine noninvasive means of evaluating platelets prior to transfusion awaits the results of ongoing clinical studies to ascertain its ability predict clinical function on transfusion.

PUBLICATIONS

Bonner, R.F.: Noninvasive Optical Assessment of Platelet Viability. U.S. Patent #4,522,494, 1985.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RS-10097-06 BEI

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Studies in Cardiovascular Dynamics

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

R.S. Chadwick, Biomedical Engineer, BEIB, DRS
J.C. Ohayon, Visiting Fellow, BEIB, DRS
C. Oddou, Professor, University of Paris
P. Brun, Senior Researcher, INSERM U. 138
B. Levy Senior Researcher, INSERM U. 141

COOPERATING UNITS (if any)

INSERM U.138 U.141
University of Paris XII, Laboratory of Physical Mechanics

LAB/BRANCH
Biomedical Engineering and Instrumentation

SECTION
Mechanical Engineering Section

INSTITUTE AND LOCATION
DRS, National Institutes of Health, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Our existing model for myocardial and ventricular mechanics will be refined to include an isotropic component of elasticity, passive and active viscoelasticity, non-cylindrical geometry, pathological fiber organization, myocardial blood flow, myocardial oxygen demand, and effects of activation pattern. The model will used in conjunction with ultrasonic kinematic data to determine performance parameters. The physiological relationships between phasic coronary arterial and venous pressure and blood flow, aortic input impedance and ventricular dimensions will be determined. A mathematical and physical model of myocardial blood flow will be developed incorporating vessel collapse. Experimental techniques will be developed to measure vascular distensibility in man. RF backscatter from the myocardium will be investigated both experimentally and mathematically with the idea of determining the stress field. Theoretical models of wave propagation in arteries and aortic input impedance will be checked against experimental data.

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OBJECTIVES: To provide a framework for determining ventricular function, both at the fiber level and for the entire structure; to relate the relationship between coronary pressure and flow to the poro-elastic rheology of the myocardium; to provide a framework for evaluating those aspects of cardiovascular function that depend on the filling of, and ejection from the left ventricle; to develop new non-invasive measurement techniques for cardiovascular research; to provide a theoretical framework for the prediction of myocardial ischemia.

METHODS EMPLOYED: An interdisciplinary approach will be utilized involving clinical ultrasound data, hemodynamic measurements on animals, physical models, and mathematical models.

SIGNIFICANCE: This research program with its interdisciplinary approach has the potential to increase fundamental knowledge in cardiovascular physiology; and to improve diagnosis and treatment of cardiovascular disease.

PROPOSED COURSE: Richard Chadwick will visit INSERM laboratories U.138, U.141 for two months to validate mathematical theories of myocardial blood flow, myocardial oxygen demand, and RF backscatter from the myocardium.

PUBLICATIONS

Brun, P., Chadwick, R.S., Levy, B. Proceedings of the NIH-INSERM Workshop on Cardiovascular Dynamics, 1985, INSERM, Paris, France (in press).

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RS 10098-06 BEI

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Laser Instrumentation for Vitreous & Cardiovascular Microsurgery

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

R.F. Bonner	Physicist	BEIB DRS
P.D. Smith	Physicist	BEIB, DRS
M. Leon	Sr. Investigator	CB, NHLBI
S. Epstein	Chief	CB, NHLBI
D. Lu	Fellow	CB, NHLBI
R. Clark	Chief	SB, NHLBI
C. Kupfer	Director	NEI

COOPERATING UNITS (if any)
Clinical Branch, NEI; Optical Science Branch, Naval Research Lab; Cardiology Branch & Surgery Branch, NHLBI;

LAB/BRANCH
Biomedical Engineering and Instrumentation

SECTION
Electrical and Electronic Engineering

INSTITUTE AND LOCATION
DRS, National Institutes of Health, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	2.5	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This program covers the long standing development of laser microsurgical techniques which initially centered on ophthalmological applications and new systems of interest in that field (Pulsed Carbon Dioxide lasers and pulsed ND:YAG slit lamp-based laser systems). Its primary focus for the last 3 years has been in cardiological application of lasers, in particular laser angioplasty. In order to develop new laser microsurigal techniques in this new field, it was necessary to analyse a variety of laser sources and their tissue effects and the feasibility of transmission through flexible fiber optics. New sources such as Erbium:YAG (YLF) lasers coupled to zirconium fluoride optical fibers and excimer lasers coupled to fused silica fibers were examined as well as more conventional sources (pulsed carbon dioxide and Nd:YAG lasers, and continuous wave argon and dye lasers).

In collaboration with the Naval Research Lab, we have developed the new eribium laser microsurgery technology through feasability studies to the development of hte initial microsurigal prototype using zirconium fluoride fibers. Refinement of this system is primarily directed towards intravascular ablation (laser angioplasty) but include application to intraocular surgery (particularly vitreal and corneal transplantation), destruction of renal stones, and endoscopic microsurgery.

Parallel development of excimer microsurgery has evaluated state-of-the-art technology and provided a research microsurgical system for in vivo studies.

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OBJECTIVES: The current program objectives are: (1) select an optimal laser source for laser angioplasty, (2) design and develop a microsurgical system using such a source, (3) evaluate the performance of these systems (with refinements) through in vitro and in vivo experiments and (4) examine other areas of clinical importance for the application of these new microsurigal tools.

METHODS EMPLOYED: Careful dosimetry of laser-tissue effects and laser-induced damage of fiber optics determine relative efficacy of the different laser sources. Tissue histological damage as well as thermal transients surrounding the ablated volume are quantitated. Variation in laser pulse parameters (wavelength, temporal sturture and spatial structure) is performed in order to characterize the physical processes involved so as to understand and predict effects. A variety of physical detection means have been used in order to evaluate effects and test models of the process of laser damage in tissue both in vitro and in vivo. Final evaluation of system design is to be performed in a variety of animal models to test performance, risks, and long term efficacy.

MAJOR FINDINGS: (1) Excimer laser ablative thresholds and slope efficiencies appear to depend on tissue absorption coefficient and material strength of the tissue (e.g., soft tissues versus calcified tissues) whereas the fiber damage thresholds depend on non-linear optical breakdown. (2) 308 nm laser pulses have been transmitted through fused silica fibers of 200 to 600 um diameter to cut tissues in vitro and in vivo. However these probes are inherently quite stiff if compatible with making a 1 mm or greater channel, and redesign of delivery systems compatible with percutaneous coranary artery angioplasty is problematic. (3) Healing of intimal and muscle wall ablation with 308 nm in the femoral artery appears satisfactory. (4) A prototype Erbium: YAG laser emitting at 2.9 um provides the ability to precisely cut soft and calcified tissues with thresholds and efficiencies comparable to excimer lasers. (5) These infrared laser pulses made be easily transmitted through prototype infrared fibers with fiber damage thresholds at least 50 fold greater than thresholds for tissue ablation. This large processing window provides the prospect for much more flexible and reliable laser catheters than those envisioned for the current excimer lasers. (6) This new prototype laser provides the potential for microsurgical advances in a variety of fields.

PROPOSED COURSE: The refinement of current prototype laser microsurgery systems to test their applicability to laser angioplasty, ophthalmic microsurgery, and destruction renal stones through in vivo and in vitro studies, proceeding where appropriate to clinical trials.

SIGNIFICANCE: Although the present mechanical vitrectomy instruments perform well in most cases, there is a risk of intraoperative complications (retinal tears and hemorrhage) when vitreal membranes are cut, especially if the membranes are taut and have a strong adhesion to the retina. "Tension" on the membranes is increased as the "cutter" of the vitrectomy instrument or the vitreous scissors cuts the tissue. This tension is transmitted to the vitreoretinal adhesion and surrounding retina predisposing this area to hemorrhage and retinal tears. If the vitreal membrane is vascularized, this tension may cause bleeding. The carbon dioxide laser vitrectomy may decrease the incidence of these intraoperative complications and increase facility in cutting selected vitreal membranes.

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The fiber optic delivery system for the CO_2 energy as well as the specific laser pulse pattern developed for optimal cutting of vitreous bands without damaging surrounding eye tissues may be generally applicable to all types of CO_2 laser surgery. The combined illumination-irrigating 20 gauge probes have improved the currently available instrumentation available for vitreous surgery.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RS 10099-06 BEI

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Cochlear Mechanics and Hair Cell Transduction

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

R.S Chadwick, Biomedical Engineer BEIB, DRS
J. Rinzel, Chief MRB, NIADDK
S. Shamma, Assistant Prof. Univ. of MD, Dept. of Electrical Engineering
K. Morrish Staff Fellow MRB, NIADDK

COOPERATING UNITS (if any)

NIADDK, Mathematical Research Branch
Univ. of Maryland, Dept. of Electrical Engineering

LAB/BRANCH
Biomedical Engineering and Instrumentation Branch

SECTION
Mechanical Engineering

INSTITUTE AND LOCATION
DRS, National Institutes of Health, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.25	0.25	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Type standard unreduced type. Do not exceed the space provided.)

A mathematical model of cochlear processing is developed to account for the nonlinear dependence of frequency selectivity on intensity in inner hair cell and auditory nerve fiber responses. The model describes the transformation from acoustic stimulus to intracellular hair cell potentials in the cochlea. It incorporates a linear formulation of basilar membrane mechanics and subtectorial fluid-cilia displacement coupling, and a simplified description of the inner hair cell nonlinear transduction process. The analysis at this stage is restricted to relatively low frequency sirgle tones. The computed responses to single tone inputs exhibit the experimentally observed nonlinear effects of increasing intensity such as the increase in the bandwidth of frequency selectivity and the downward shift of the best frequency. In the model the first effect is primarily due to the saturating effect of the hair cell nonlinearity. The second results from the combined effects of both the nonlinearity and the inner hair cell lowpass transfer function. In contrast to these shifts along the frequency axis, the model does not exhibit intensity dependent shifts of the spatial location along the cochlea of the peak response for a given single tone. The observed shifts therefore do not contradict an intensity invariant tonotopic code.

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OBJECTIVES: To calculate the electrical signals being transmitted to the brain via the 8th auditory nerve in response to pure and complex sound input in the cochlea.

METHODS EMPLOYED: The appropriate equations of fluid mechanics, solid mechanics, and neurophysiology are solved in a cascaded model of the basilar membrane mechanics, fluid-cilia coupling, and hair cell transduction. A flow frequency theory is used with corrections for 3 dimensional fluid flow effects.

MAJOR FINDINGS: The processing of a single tone has been computed. The calculated dependence of AC and DC hair cell potentials on frequency and stapes amplitude is in general agreement with reported experimental findings. We found that the DC hair cell tuning curve is sharpened by the nonlinear cilia displacement - conductance relation for the hair cell.

SIGNIFICANCE: This is one of the first attempts to coordinate what has been until now two distinct areas of investigation: cochlear mechanics and auditory neurophysiology. Such a combined approach will help to answer some fundamental questions in auditory physiology and in the design of cochlear prostheses, such as the relative importance and interaction of mechanical and neural events in auditory analysis and the relative importance of temporal versus frequency information.

PROPOSED COURSE: We plan to extend the applicability of the model to higher frequency, to continue to analyze the processing of more complex input signals; and to include the effects of an active micromechanical model with feedback onto the basilar membrane.

PUBLICATIONS

Shamma, S.A., Chadwick, R.S., Morrish, K., Rinzel, J.: A biophysical model of speech processing in the cochlea. To appear in Proceedings of 1986 IUPS Satellite Conference, Univ. of California, San Francisco 1986.

Chadwick, R.S.: Three dimensional effects on low frequency cochlear mechanics. Mech. Res. Comm., 12 (4): 181-186, 1985.

Shamma, S.A., Chadwick, R.S., Wilbur, W.J., Morrish, K.A., Rinzel, J.: A biophysical model of cochlear processing: intensity dependence of pure tone response, J. Acoust. Soc. Am. (in press) 1986.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RS 10103-06 BEI

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Triple Laser-Multi Parameter Flow Cytometry System for Study of Tumor Cell Kinetics

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

W. Schuette	Chief, ACES	BE1B, DRS
S. Shackney	Allegheny Gen. Hosp.	Pittsburgh, PA 15212
J. Dvorak	LPD-NIAID	
F. Plowman	ACES	BEID, DRS

COOPERATING UNITS (If any)

DRS-NCI
LPD-NIAID

LAB/BRANCH
Biomedical Engineering and Instrumentation

SECTION
Applied Clinical Engineering

INSTITUTE AND LOCATION
DRS, National Institutes of Health, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A Triple laser flow cytometer has been developed so that various immuno-fluorescent labeling techniques may be employed for the investigation of cell kinetics. Three laser beams at different wave lengths are made to intersect a tumor cell flow stream passing through a quartz cuvette so that multi-parameter signals may be obtained. These signals are processed by specialized electronics and then analyzed by means of a PDP 11 computer. Simplified optics have increased light detection efficiency by an order of magnitude. The system is currently being used for the investigation of a unicellular protozoon known as trypanosoma cruzi, the causative agent of Chagas disease. Electronic cell volume capability has been added to the system to assist in the bivariate analysis of trypanosoma cruzi fluorescence data.

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PUBLICATIONS:

Schutte, W.H., Carducci, E., Marti, G.E., Shackney, S.E. and Eden, M.: The relationship between mean channel selection and the calculated coefficient of variation. Cytometry 6:487-491, 1985.

Schuette, W. H., Shackney, S.E. and Marti, G.E.: Count-dependent filter for smoothing bivariate FCM histograms. Cytometry. 7:274-280, 1986.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RS 10109-06 BEI

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Adjunctive Heat Treatment of Cancer

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

R.L. Levin, Biomedical Engineer, BEIB, DRS
M. Hagmann, Senior Staff Fellow, BEIB, DRS
J-L Guerquin-Kern, Fogarty Fellow, BEIB, DRS
C. Charny, Guest Worker, BEIB, DRS
H. Coldefy, Fogarty Fellow, BEIB, DRS
A. Zabel, Physician, ROB, NCI
E.J. Glatstein, Chief, ROB, NCI

COOPERATING UNITS (If any)

Radiation Oncology Branch, NCI

LAB/BRANCH
Biomedical Engineering and Instrumentation Branch

SECTION
Mechanical Engineering Section,

INSTITUTE AND LOCATION
DRS, National Institutes of Health, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.2	4.2	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this project is to facilitate the development of adjunct hyperthermia modalities for cancer treatment by theoretically and experimentally studying the spatial and temporal variation in the temperature field of tissues subjected to microwave and radio-frequency electromagnetic radiation. Currently, we are (1) experimentally measuring the patterns of energy deposition within various types of extremity phantoms produced by a Mini-Annular Phased Array (MAPA) applicator; (2) theoretically describing the electromagnetic fields of a MAPA in terms of its design paramters; (3) theoretically describing the transient thermal profiles within limbs produced by a MAPA; and (4) theoretically describing the systemic temperature and cardiac changes associated with heating various regions of the body. We are also performing electromagnetic and thermal modeling of the unwanted non-local energy deposition occuring outside the bounds of typical hyperthermia applicators. These studies are forming the basis for the clinical system currently under construction.

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OBJECTIVES:

(1) To optimize the energy patterns within human extremities produced by a MAPA.

(2) To continue to develop a generalized mathematical model which will predict the spatial and temporal variation of the temperature field within a tissue or organ subjected to electromagnetic irradiation.

(3) To develop optimal adjunct hyperthermia modalities.

METHODS EMPLOYED:

(1) Mathematical modeling of the radio-frequency and thermal characteristics of various types of phantoms and human extremities subjected to RF irradiation from various types of applicators will be accomplished through the use of ROB's VAX-11/750 computing system and its associated graphics hardware and BEIB's VAX-11/750 computing system.

(2) Experimental measurements of the electric field and thermal profiles of various types of phantoms subjected to RF irradiation from various types of applicators will be accomplished through the use of a dedicated PC/AT computing system and its associated data acquisition system and also a BSD-200 Thermal Monitoring Subsystem. Such a system will form the basis of the clinical system under construction.

MAJOR FINDINGS:

The energy deposition patterns within several isolated human legs heated with a mini-annular phased array (MAPA) hyperthermia applicatorwere determined. Typically, the non-tumor bearing lower portion of a human leg which had been therapeutically amputated at the hip due to the presence of a large tumor in the thigh was "fixed" in a 50% ethanol in 0.9% saline solution. Subsequent to this fixation process, the leg was rehydrated in 0.9% saline and heated using a MAPA. One leg was also heated within hours of being amputated without being fixed. Specific absorption rates and electric field strengths were calculated from the rates of change of temperature with time measured at 143 different anatomical locations within each leg. When the legs were coaxial with the MAPA and the MAPA was axially positioned midway between the knee and the ankle, the points of maximum heating were skewed away from the center of the MAPA, towards the ankle of the legs and along the central axis of the MAPA. Significant temperature rise was measured inside the bone and fat as well as inside the muscle of the legs. Coupled with low blood flow to the bone, this condition could lead to excessively high temperatures inside the bone. The frequently applied assumption that SAR in bone is negligible may be incorrect. Our ability to selectively heat a particular region with a MAPA was shown to be limited by physical constraints on the movement of the leg within the MAPA. This problem may be overcome with modifications which allow for amplitude and/or phase shifting of the individual dipole elements. Such changes would facilitate more predictable focusing of energy deposition to a desired location in the treated limb and are currently being studied.

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SIGNIFICANCE: At present, the heat treatment of cancerous cells when combined with conventional radiotherapy and chemotherapy shows considerable promise in the management of cancer. Nevertheless, there still remain numerous important problems that must be resolved. Of paramount importance is the problem of generating and controlling uniform temperature fields within tissues. This study will therefore attempt to facilitate the development of optimum hyperthermia modalities by theoretically and experimentally studying the temperature fields within tissues subjected to radio frequency EM radiation.

PROPOSED COURSE:

(1) To continue the electromagnetic and thermal characterization of various phantom applicator combinations using non-perturbines probes.

(2) To develop a clinical hyperthermia system using a MAPA which can be phased and amplitude shifted so that the energy deposition pattern can be varied.

PUBLICATIONS:

Hagmann, M.J., Levin, R.L.: Accuracy of block models for evaluation of the deposition of energy by electromagnetic fields. IEEE Trans., MIT, 34: 653-659, 1986.

Hagmann, M.J., Levin, R.L.: Aberrant heating: a problem in regional hyperthermia. IEEE Trans., Biomed. Eng., 33: 405-411, 1986.

Hagmann, M.J., and Levin, R.L.: Convergence of local and average values in three dimensional moment method solutions. IEEE Trans., MIT 33: 649-654, 1985.

Hagmann, M.J. and Levin, R.L., and Turner, P.F.: A comparison of the annular phased array with helical coil applicators for limb and torso hyperthermia. IEEE Trans., Biomed. Eng., 32: 916-927, 1985.

Guerquin-Kern, J-L., Hagmann, M.J., and Levin, R.L.: Experimental characterization of the helical coil as a hyperthermia applicator. Submitted to IEEE Trans., Biomed. Eng., 1985.

Guerquin-Kern, J-L, Hagmann, M.J., and Levin, R.L.: Experimental characterization of the BSD MAPA. Submitted to Medical Physics, 1986.

Charny, C., Guerquin, J-L, Hagmann, M.J., Levin, S.W., Lack, E.E., Sindelar, W.F., Zabell, A., Glatstein, E.J., and Levin, R.L.: Human leg heating using a MAPA. Medical Physics, 13: 449-456, 1986.

Hagmann, M.J., Levin, R.L.: Procedures for non-invasive electromagnetic property and dosimetry measurements. Submitted to Medical Physics, 1986.

BEST COPY AVAILABLE

Hagmann, M.J., and Levin, R.L.: Non-invasive determination of local energy deposition from measurements of near fields. (to be included in Near field Microwave Imaging. J.J. Wang, (Ed.), Scheduled to appear in 1986).

Charny, C., Guerquin-Kern, J-L., Hagmann, M.J., Levin, S.W., Lack, E.E., sindelar, W.F., Zabell, A., Glatstein, E.J., and Levin R.L.: Human leg heating using a MAPA. Proceedings of 1986 NAHG Meeting, 1986.

Hagmann M.J., Guerquin-Kern, J-L., and Levin, R.L.: Control of the energy deposition pattern from a MAPA by adjustment of phase and amplitude. Proceedings of 1986 NAHG Meeting, 1986.

Hagmann, M.J. and Levin, R.L.: Non invasive determination of local energy deposition during hyperthermia. Proceedings of 1986 NAHG Meeting, 1986.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RS 10110-06 BEI

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
A Dual 3-Dimensional Position Monitor For Speech Analysis: MOD. II

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

E.C. Walker, Mechanical Engineer, ACES BEIB

Other Investigators
T.L. Talbot, Mechanical Engineer ACES BEIB
C.L. Ludlow, LCD NINCDS
F. Plowman, ACES, BEIB

COOPERATING UNITS (if any)
LCD-NINCDS

LAB/BRANCH
Biomedical Engineering and Instrumentation

SECTION
Applied Clinical Engineering

INSTITUTE AND LOCATION
DRS, National Institutes of Health, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.25	.25	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Several speech monitoring instruments have been designed to study the etiology of normal and abnormal articular movements in humans. The first system, which is in current use, provided a unique method of monitoring 3-D lip and jaw movements. The second system, MOD II, is an improved version of the first. The device consists of two mirror image transducers mounted on a common head frame. Each transducer, which can be individually adjusted, is capable of measuring the movement of a point in three orthogonal planes. The transducers have been structurally and operationally redesigned to provide increased linearity, resolution, and reduced weight. Additionally, the head frame has been totally redesigned to provide improved wearing comfort and reduced weight. The improved system will facilitate studying a broader patient population such as children and the elderly.

Design and fabrication of MOD II has been completed. Results of bench tests indicate that the system performs well, within its specified range. Future efforts will be directed toward patient testing.

Following system enhancement, patient testing will be scheduled for the upcoming fiscal year.

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Due to the patient population (which includes dysarthric patients) that this device is designed for, future enhancements will include improved resolution along the lateral-medial axis and reduction of inertial response.

PUBLICATION

Walker, E.C., Talbot., Doram-Quine, M.E., Ludlow, C.L.: Monitoring mouth motion, SOMA (in press) 1986.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RS 10112-06 BEI

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Analysis of Microcirculatory Blood Flow by Laser Doppler Scattering

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)
R.F. Bonner, Physicist, BEIB, DRS
T.R. Clem, Elect. Engineer, BEIB, DRS
M. Kaliner, Section Chief LCI NIAID
H. Druce, Clinical Assoc. LCI NIAID
G. Rogers, Clinical Assoc. LCI NIADDK
M. Roy, Visiting Scientist NEI
D. Goldstein, Sr. Invest. HB NHLBI; H. Kaiser, Chief HB NHLBI
E. Oldfield, Neurosurgeon SN NINCDS; R. Blacklock, Clinical Assoc. SN NINCDS

COOPERATING UNITS (if any) HB, NHLBI; CB NEI; LCI, NIAID; ROB, NCI; SN, NINCDS; LAP, DCRT; MedPacific, Inc, Seattle, WA; TSI, Inc, St. Paul, MI; International Diabetes Center, Minneapolis, MI; Neurology and Gastroenterology Depts., USUHS, Bethesda, MD, Dept. Medicine, Med. Coll. S. Carolina, Charleston, SC.

LAB/BRANCH
Biomedical Engineering and Instrumentation

SECTION
Electrical and Electronic Engineering

INSTITUTE AND LOCATION
DRS, National Institutes of Health, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project has developed a clinically useful method (Laser Doppler instrument and its theory of operation) for clinical measurements of microcirculatory blood flow, number density of flowing RBC's, and mean RBC velocity. Assistance has been given to the commercialization of this methodology and its application to clinical research in many laboratories worldwide. At the NIH our clinical studies have been directed to examining normal and abnormal microvascular dynamics through noninvasive clinical studies (of skin and nasal mucosa) and in intra-operative clinical studies (of muscle and CNS). Collaboration with USUHS is directed at developing endoscopic application of this technology. Considerable theoretical work has been directed towared an adequate construct with which to intercept the physical measurements and refining the accuracy of the microcirculatory measurements. We have discovered abnormal microcirculatory patterns and responses in the skin of patients with sickle cell disease, hypertension, certain cardiac circulatory syndromes, diabetes and skin cancer. The microcirculatory effects of therapy are monitored with this technique in these patients affording a better understanding of the microcirculatory component of these diseases.

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OBJECTIVES: To develop our understanding of the human microcirculation in health and disease and to refine the clinical utility of lase Doppler measurements of the microcirculation.

METHODS EMPLOYED: Application of the laser Doppler flowmeters to various clinical protocols is based on the understanding of the technique and of microcirculation of the targeted tissue. As very little clinical work has here-to-fore been performed on microcirculatory dynamics, protocols are frequently designed to detect highly significant abnormalities and then to direct the protocol towards the characterization of these abnormalities. Theoretical modeling of the signal and, to a limited degree, of the microcirculation facilitates the intrepretation of the microcirculatory measurements.

MAJOR FINDINGS: (1) The blood volume and mean RBC velocity signals as well as the blood flow signals obtained by the laser Doppler method developed at the NIH provide reproducible and clinical useful measurements. (2) A light diffusion theory and empirical optical measurements has better defined the sample volume and the absolute calibration of the measurements for a variety of tissues. (3) Myogenic vasomotion in a variety of human tissue can be easily detected and quantified. This activity is dramatically increased in a variety of diseases and may play a critical role in microcirculatory adaptation to microvascular or rheological abnormalities. (4) Measurements in the CNS of patients undergoing surgery can be made reliably with hand help probes and appears useful in analyzing the local microvascular response during AVM surgery. (5) Skin blood flow measurements can be used to quantify sympathetic innervation in patients with surgical sympathectomy and presumably in those with neuropathy in a noninvasive measurement. (6) The measurement of microcirculatory response to Photodynamic therapy of tumors has been evaluated during and following therapy.

PROPOSED COURSE: Pursue clinical protocols to establish and evaluate the contribution of microcirculatory abnormalities in a variety of diseases. To refine the technology so as to facilitate laser Doppler measurements in a variety of tissues in particular those endoscopically assessable. To continue to assist commercial manufacturers with the application of this technology to continue to assist commercial manufacturers with the application of this technology to accurate, reliable and useful commercial clinical instruments.

SIGNIFICANCE: The NIH-developed technology for measuring tissue microcirculatory blood flow increasingly is gaining international acceptance as a powerful clinical research tool. The specific clinical studies performed in this project already have opened new directions in research into a variety of diseases such as sickle cell disease, hypertension, scleroderma, diabetes and allergy.

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PUBLICATIONS:

Druce, H.M., Bonner, R.F., Choo, P., Patow, C., Summers, R., and Kaliner, M.A.: Response of nasal blood flow to neurohormones as measured by laser-doppler velocimetry. J. Appl. Physiol. 57: 1276-83, 1984.

Rodgers, G.P., Schechter, A.N., Noguchi, C.T., Klein, H.G., Nienhius, A.W., and Bonner, R.F.: Periodic microcirculatory flow in patients with sickle cell disease. N. Engl. J. Med. 311 :1534-8, 1984.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RS 10116-05 BEI

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Modeling of Arterial Pulse Waves

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

R.S. Chadwick, Biomedical Engineer, BEIB,DRS
D. Goldstein, Senior Investigator EHL, NHLBI
H. Kaiser, Chief EHL, NHLBI

COOPERATING UNITS (if any)
NHLBI, Endocrine-Hypertension Laboratory

LAB/BRANCH
Biomedical Engineering and Instrumentation

SECTION
Mechanical Engineering Section

INSTITUTE AND LOCATION
DRS, National Institutes of Health, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.25	.25	

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A pulse wave theory and a model of the human brachial arterial system is developed that predicts the changes in the arterial pressure waveforms as it traverses the vasculature - increased pulse, sharper main wave, disappearance of the aortic incisura, and appearance of a diastolic dicrotic wave. It also predicts the observed modulation of the waveform during phenylephrine - induced vasoconstriction and nitroglycerin - induced vasodilation. The model considers the brachial arterial system as a tapered distensible tube ending in a loop, with side branch networks represented by distributed Windkessels, and it uses verifiable values for realistic parameters. We found that the vertical modulation of the dicrotic wave in people decreased with advancing age and with high blood pressure, the model explaining these finding in terms of increasing vascular rigidity and decreasing small vessel vasodilator responsiveness. We noted a significant negative correlation between the arterial level of plasma norepinephrine and the amount of modulation of the dicrotic wave after nitroglycerin among subjects 40 years old or younger, suggesting a sympathetic neurogenic contribution to the vascular abnormalities observed in relatively young patients with essential hypertension.

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OBJECTIVES: To establish the mechanisms that are responsible for the clinically observed reduced dicrotic wave modulation of young hypertensive subjects in response to vasoactive drugs.

METHODS EMPLOYED: The new theory developed to deal with wave propagation in a branching system has been implemented for a efficient numerical computation of pressure and flow waveforms as well as frequency analysis of vascular impedance. The basic computation is done in the frequency domain, and periodic waveforms are computed using Fourier synthesis. The optimal parameter set is obtained by a computer algorithm.

MAJOR FINDINGS: We found that very realistic pressure waveforms can be computed with this theory. To simulate the clinically observed modulation of the dicrotic wave, it is necessary to change both the main branch wave speed and the side branch network resistance and compliance.

SIGNIFICANCE: The theoretical work described here leads to the suggestion that defective modulation of the dicrotic wave with age and high blood pressure result from a combination of increased large vessel stiffness and decreased vasodilator capacity. Such a finding helps to identify both the sites and modes of action of vasoactive drugs as well as clarifying the fundamental processes in the control of blood pressure. The approach used in the present study represents one of the first attempts to use a circulatory model to explain abnormalities in the contour of the pressure waveform in hypertension.

PROPOSED COURSE: Continued measurements of brachial artery blood flow and arterial distensibility are planned to further validate our present views on dicrotic wave modulation.

Plans for a completely noninvasive protocol are being formulated that utilize a Doppler flow pulse wave rather than the pressure wave.

PUBLICATIONS

Chadwick, R.S., Goldstein, D.S., Keiser, H.R., McGuire, D.A.: Pulse-wave model of brachial arterial pressure modulation in aging and hyperthermia, Am. J. Physiol, (251) (Heart Circ. Physiol 20), H1-H11, 1986.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RS 10122-05 BEI

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Micro-computer Applications for the NIH Bio-Technology Unit (Pilot Plant)

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

T.R. Clem, Sr., Electronics Engineer, EEES, BEIB, DRS

Other Investigators:

Y. Shiloach	Chief, Bio-Technology Unit	LCDB NIADDK
A. LeRoy	Chemical Engineer	BEIB, DRS

COOPERATING UNITS (If any)

LCDB - NIADDK

LAB/BRANCH
Biomedical Engineering and Instrumentation Branch

SECTION
Electrical and Electronic Engineering Section

INSTITUTE AND LOCATION
DRS, National Institutes of Health, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	0.75	0.25

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The NIH Bio-Technology Unit (Pilot Plant) has various instrumentation needs that have not been previously addressed or are currently using cumbersome techniques and/or outdated equipment. This project has been concerned with satisfying these instrumentation needs using modern equipment and low cost desktop size Personal Computers. Several PC's have been acquired and are being installed to supply a variety of services which include process control, data acquisition, data analysis, and administrative functions. The instrumentation and process control functions utilize commercial equipment, such as possible, and are interfaced using standard I/O connections such as the IEEE-488 GPIB and the RS-232 Serial I/O ports.

Using these techniques allows changes in the parameters measured or controlled, or scaling to different size vessels to be accomplished relatively quickly and easily. Utilizing the computational capabilities of the computer/controller allows initial selection of the operating parameters and dynamic alteration of these parameters as the process continues, thus allowing optimization of yields or detailed study of the process parameters.

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OBJECTIVE: To Increase efficiency and productivity of the Bio-Technology Unit, to expand the fermentation process capability, and to develop methods for researching the fermentation process through the expanded use of inexpensive desktop Personal Computers.

METHODS EMPLOYED: Desktop Personal Computers and interface electronics, both commercial and custom designed, are connected to both new and previously owned laboratory instruments and to fermentation vessels via the IEEE-488 GPIB, the RS-232, or direct connections. Interfacing techniques are kept as versatile as practical to allow system changes to be accomplished relatively easily. The control programs are written in a high level language, such as BASIC or Pascal, to allow program changes to be accomplished easily. All data is stored in a general form (ASCII) so that most data analysis programs may access the data.

SIGNIFICANCE: Computer monitoring and controlling of the fermentation process produces several significant advantages over manual methods. By using the computer to make decisions based on what is occurring in the fermentation process, parameters can automatically be altered to either produce an increased yield of a particular product, produce a purer form of the product, or study the effects on a particular product of the alteration of selected parameters. The computer can also perform some of the "housekeeping" tasks associated with running a fermentation process that would normally occupy an operator. Complete records of any run are saved on disk storage for later analysis. The computers aquisition, control, and computational capabilities allows growth rate and other parameters to be assessed under a variety of environmental conditions. The presence of a computer in the Bio-Technology Unit provides administrative support in the form of records-keeping and text-editing. This has taken on new importance in the light of recent concern over pilot safety which requires detailed logs of materials grown in the Pilot Plant.

PROPOSED COURSE: To continue expanding and refining the system to provide additional functions, monitor and control additional parameters, and implement more sophisticated control programs to expand operation of the system. Larger fermentation vessels with more modern control systems are being acquired by the pilot plant and these will be interfaced with our current systems.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RS 10132-05 BEI

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Image Processing and Cell Classification

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

J.R. Ellis	Expert	BEIB, DRS
C.C. Gibson	Electronics Engineer	BEIB, DRS
M.A. Greenwood		SNB, NINCDS
G.H. Hook		SNB, NINCDS

COOPERATING UNITS (If any)
SNB, NINCDS

LAB/BRANCH
Biomedical Engineering and Instrumentation Branch

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DRS, National Institutes of Health, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.25	0.15	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In vitro cell culture is an effective method for the study of the effects of chemotherapeutic agents on neurological tumors. Quantitation of all but the most rudimentary of these effects is generally beyond the ability of human observers. Thus, an automated system for acquisition of image data, extraction of parameters of interest, and statistical processing of results is necessary for any quantitative effectiveness.

Historically, this field has been qualitative. Thus, moving toward quantitative evaluations has involved a very broad spectrum of discussions about methods, limitations of methods, desired results, and significance of results. In addition, it has been necessary to clarify the strong and weak points of human and automated image processing, and to determine those problems which require a combination of the two methods.

Morphology studies using size, aspect ratio, and a shape factor were begun some time ago on micrographs of granules and mitochondria of Glioma tumor cell lines treated in-vitro with AZQ, BCNU, and other drugs. These studies have recently been resurrected.

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OBJECTIVES: Quantitative analysis of morphological properties of in-vitro Glioma cell cultures under varied conditions and courses of treatment. Most recently, the study of mitochondrial response has been emphasized.

METHODS: Cell lines are taken from surgical biopsies and cultured by standard methods. Sample cultures are subjected to selected therapeutic procedures. Photographic prints of electron microscopic images of both control and treated cell populations are analyzed by digitizing operator-recognized and defined outlines of mitochondria using a Numonics digitizer. Standard descriptive parameters, such as area, perimeter, and shape factors are calculated using standard polygonal approximation formulas.

MAJOR FINDINGS: Meaningful quantitative results can be obtained in this environment. More importantly, even though desired features cannot always be recognized automatically, it has been found that use of intermediary representations, such as overlays, can be useful match between the complex human visual recognition system and the detailed computational world of the machine.

Size, length-to-breadth ratio, and perimeter-to-(square root of area) ratio have been measured on photomicrographs of granules and mitochondria. Initial results are consistent with qualitive observations of drug effects - e.g., the effects of AZQ on mitochondria.

SIGNIFICANCE: Reliable, consistent, quantitative processing of image data concerning neurological tumors must be done by machine. The history of operator fatigue and boredom in these tasks is well known. Further, humans can seldom make any quantitative measurements beyond cell counts. The program of study underway here can widen the range of feasibility of investigations far beyond the qualitative arena which now exists.

PROPOSED COURSE: We propose to continue using the Numonics digitizer and polygonal approximation to perform computer-assisted digitization of the mitochondrial images. These digitized images will then be used to seek quantitative descriptions of relationships among cell lines and courses of treatment.

PUBLICATIONS:

Smith, B.H., Greenwood, M.A., Kornblith, P.L., Ellis, J., Gibson, C., and Cummins, C.J., "Non-nuclear cytottonic actions of DNA cross-linking and/or alkylating agents in glioma-derived cell lines". Poster presentation, Second International Symposium on the Biology of Brain Tumours, London, UK, October, 1984.

Smith, B.H., Greenwood, M.A., Cummins, C.J., Ellis, J., Gibson, C., Kornblith, P.L., "Non-nuclear Cytotoxic Actions of DNA Cross-Linking and/or Alkylating Agents in Glioma-Derived Cell Lines". Submitted to Journal of NeuroOncology.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RS 10146-04 BEI

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Prosthetic Urethral Sphincter

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

S. B. Leighton, Mechanical Engineer, BEIB, DRS,
Steven Scoog, M.D. WRAMC
T. Rade Baugh, BRCM, NIA

COOPERATING UNITS (if any)
BRCM, NIA
DCT, NCI

LAB/BRANCH
Biomedical Engineering and Instrumentation Branch

SECTION
Mechanical Engineering Section,

INSTITUTE AND LOCATION
DRS, National Institutes of Health, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.05	.05	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A number of techniques are used for treating urinary incontinence, including a number of artificial prosthetic sphincters. The present work concerns an entirely intraurethral artificial sphincter that can be implanted without surgery. Concepts are being explored which would allow the device to be used in situations in which surgery is contraindicated, and would also presumably lower the cost. The valve would be appropriately matched to urethral dimensions, pressures, and flowrates. The valve would be potentially useful in cases of nonopening normal valves as well as in cases of non-closing valves.

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OBJECTIVES: To develop and test an artificial prosthetic urethral sphincter that can be introduced non-surgically into the urethra.

METHODS: A large scale model of an artificial valve with appropriate pressure flow characteristics has been designed. It will be bench tested and miniaturized. After thorough and successful bench testing, clinical tests will be designed to verify function and safety.

MAJOR FINDINGS: Potential collaborators for fabrication of the device were identified and approached.

SIGNIFICANCE: Urinary incontinence affects about 10% of the population over 65, and significant numbers of younger people due to paralysis, infection, and other causes. A simpler, less expensive treatment technique would presumeably be useful for at least a reasonable fraction of these cases.

PROPOSED COURSE: Design and bench testing of various valve designs will proceed. Clinical tests will be designed to test proper function and safe operation.

U.S. Patent #4,553,533 issued for this device 11/85

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RS 10151-04 BEI

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Nuclear Magnetic Resonance Imaging

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Dr. David I. Hoult, Physical Scientist, BEIB, DRS

Other personnel:
Dr. Ching-Nien Chen, Expert
Dr. Victor J. Sank, Expert
Dr. L. Kyle Hedges, Staff fellow

COOPERATING UNITS (if any)
Department of Radiology, Clinical Center

LAB/BRANCH
NMR Imaging Laboratory, Biomedical Engineering and Instrumentation

SECTION
Office of the Chief

INSTITUTE AND LOCATION
DRS, National Institutes of Health, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	2.8	0.2

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects
  - ☐ (a1) Minors
  - ☐ (a2) Interviews
- ☐ (b) Human tissues
- ☒ (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A considerable amount of work has been put into the rf probe design for the 3-dimensional rotating frame NMR imaging method, at 5 MHz, as an extension from our previous 2D projects. Because of limitation in geometry, the coils interact strongly rendering impossible individual tuning and matching by conventional means. We have successfully devised circuits to overcome these difficulties. The same principle can be utilized in other similarly coupled rf coils. Existing softwares are modified and expanded to accommodate the new hardware for data collection and reduction.

A FORTRAN program package is being developed to give numerical solutions to the Bloch-Riccati equations for arbitrary rf pulses.

A preliminary investigation has been conducted with regard to spectral treatment by the maximum entropy method, which has been introduced to the NMR field recently to improve the signal-to-noise ratio.

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OBJECTIVES: The development of new techniques and the optimization of instrumentation for nuclear magnetic resonance imaging.

METHODS EMPLOYED: The blocking mechanism necessary for stopping the interactions between the rf coils is achieved by using PIN diodes which switch rf coils rapidly between a tuned and matched state and a high-impedance blocking state. Blocking current in a coil effectively decouples it from all other coils. The revised circuit and software were proved to be functioning well by successful 2D test experiments.

PROPOSED COURSE: To continue work on 3D rotating frame imaging method using the revised hardware and software. A program package, which gives numerical solutions to the Bloch-Riccati equations, will be implemented on the VAX 11/750 together with an array processor AP500. To continue the maximum entropy method implementation for 1D and 2D data.

PUBLICATIONS

Chen, C-N., Sank, V.J., Cohen, S.M., Hoult, D.I.: The field dependence of NMR imaging. I-laboratory assessment of signal-to-noise ratio and power deposition. Mag. Reson. Med. 3 1986.

Hoult, D.I., Chen, C-N., Sank, V.J.: The field dependence of NMR imaging II - arguments concerning an optimal field strength. Mag. Reson. Med. 3, 1986.

Sank, V.J., Chen, C-N., Hoult, D.I.: A quadrature coil for the adult head. J.Magn. Reson., 69, pp. 236-242, 1986.

Hoult, D.I.: Sensitivity of the NMR imaging experiment. in "Proceedings of the NATO Advanced Study Institute on NMR in Living Systems, Sicily, Italy. Aug. 26 - Sep. 7, 1984." pp. 117-122 (1986) Eds. T. Axenrod and G. Ceccarelli.

ABSTRACTS

Hedges, L.K., Hoult, D.I.: A probe for 3D Rotating Frame Zeugmatography. 27th Experimental NMR Conference, Baltimore, Md. 1986.

Hedges, L.K., Hoult, D.I.: Isolation of Non-Orthogonal Radio-Frequency Coils for NMR. p. 1096, Program abstract of the 4th annual meeting of the Soc. Magn. Reson. Med., Aug. 19-23, 1985 London, UK.

Kan, L.S., Chen, C-N.: Implementation of MEM on a spectrometer system. 27th Experimental NMR Conference, Baltimore, Md. 1986.

INVITED PRESENTATIONS

D. Hoult

European NMR Conference,
University of Florida, Gainsville, FL
The Johns-Hopkins University, Baltimore, MD.

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C-N Chen

The Johns-Hopkins University, Baltimore, MD.
The Johns-Hopkins University, Baltimore, MD.

K. Hedges

27th Experimental NMR Conference, Baltimore, MD.

V.J. Sank

The Johns-Hopkins University, Baltimore, MD.
DCRT, NIH Student Elective Course
Georgetown University Medical School

PROFESSIONAL ACTIVITY

D.I. Hoult was on sabbatical leave in Grenoble, France from March to July 1986, gave multiple teaching lectures in NMR techniques and conducted experiments in the lab of professor P. Servoz-Gavin.

AWARD

D.I. Hoult was awarded the 1985 gold medal of the Society of Magnetic Resonance in Medicine.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RS 10156-04 BEI

47

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Differential Scanning Calorimeter

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

C.P. Mudd, Biomedical Engineer, ACES, BEIB, DRS
T. Talbot, Mechanical Engineer, ACES, BEIB, DRS
P.D. Ross, Physical Chemist, A LMB, NIADDK

COOPERATING UNITS (if any)
A LMB, NIADDK

LAB/BRANCH
Biomedical Engineering and Instrumentation

SECTION
Applied Clinical Engineering

INSTITUTE AND LOCATION
DRS, National Institutes of Health, Bethesda. MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The application of sensor design techniques from earlier work in heat conduction calorimeters to scanning calorimeter design has resulted in a scanning calorimeter which has a 10-fold increase in its effective sensitivity. Unfortunately, the high temperature scanning associated with this calorimeter has produced unexplained and random failures in the thermopiles which create an unacceptable increase in the baseline noise. Since these sensors are manufactured for use as heat pumps (Peltier mode of operation) and not sensors (Seebeck mode of operation), the manufacturers can supply no information on expected noise performance. We have therefore initiated a program to evaluate the noise and failure modes of these devices from different manufacturers. In the process of evaluating these devices, we will also measure their Seebeck sensitivity as an aid in matching the channel sensitivities in the scanning calorimeter.

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OBJECTIVES The original objective remains unchanged: To scan or change the temperature of a sample and observe the small changes in the heat content of the sample which are attributable to physical changes within the sample, (e.g. conformational changes in proteins) and not to changes in the heat content due to the scanning rate. To accomplish this with minimum system noise, we must evaluate several different sources of the thermopiles in order to determine their noise characteristics and failure modes at high temperatures. The manufacturers are unable to supply this information.

SIGNIFICANCE: The identification of the failure modes and the source of the noise which appears after several high temperature runs will enable us to more fully evaluate the use of these sensors in Differential Scanning Calorimetry.

PROPOSED COURSE: We have currently identified four different manufacturers of the sensors which could be used in the DSC. As a first screening step, we will use a computer controlled testing system to cycle the transducers over the temperature range of operation, measure the noise as a function of time and temperature, and determine the sensitivity of each unit. Based upon the results of these tests, we will mount the most suitable transducers in the existing two channel DSC as a final evaluation before completing the four channel DSC.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RS 10157-03

PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Temperature Controlled Chamber for X-ray Diffraction Specimens

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

C.P. Mudd, Biomedical Engineer, ACES, BEIB, DRS
H.W. Tipton, Mechanical Engineering Tech., ACES, BEIB, DRS
A.V. Parsegian, Researcher, CR, PSL
B.K. Lee, Researcher, CR, PSL
D. Rau, A, LCP

COOPERATING UNITS (if any)

DCRT. Cr-PSL

LAB/BRANCH

Biomedical Engineering and Instrumentation

SECTION

Applied Clinical Engineering

INSTITUTE AND LOCATION

DRS, National Institutes of Health, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A prototype chamber was constructed of polycarbonate and aluminum with a beryllium window to allow the entry of the x-ray beam. The cell containing the specimen is loaded into a holder next to the window. The bottom of the holder is held in contact with one side of a Peltier solid state heat pump. The opposite side of the heat pump is clamped to the aluminium bottom of the chamber which serves as the heat sink. In this early version, a thermistor was used to control the heat pump to keep the specimen at the set temperature. The film plate is mounted inside the chamber on an adjustable slide. Based on the success of this prototype, two more chambers were requested with different focal lengths. The new units were designed with an improved temperature controller. The new unit incorporates a linear feedback sensor with a bipolar supply to allow automatic switching from cooling to heating. The set temperature is fixed by a 3 digit thumbwheel switch and the sample temperature is read out on a $3\frac{1}{2}$ digit display. This new sytem will hold the sample temperatures between -3°C and 85°C with a stability of 0.02° C.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RS 10159-05 BEI

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Pathophysiology of Cachexia in Sarcoma Patients

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)
R. Corsey, Electronic Engineer BEIB, DRS

J.A. Norton, Section Chief, SURG, NCI
R. Inculet, Research Associate, NCI

COOPERATING UNITS (if any)
Nutrition Department,Clinical Center, NIH
Nursing Department, Clinical Center, NIH

LAB/BRANCH
Biomedical Engineering and Instrumentation

SECTION
Applied Clinical Engineering

INSTITUTE AND LOCATION
DRS, National Institutes of Health, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.5	0.5

CHECK APPROPRIATE BOX(ES)

- [X] (a) Human subjects
  - [X] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This study is designed to explore alterations in metabolic demand which sarcomas imposed on patients. Since soft-tissue sarcomas have been shown to be metabolically active tumors and NCI is treating a large number of patients with extremity and truncal sarcomas this study will be limited to sarcoma patients. Of particular interest is the following:

1. Is there any alteration in resting energy expenditure due to the presence of the tumor?

2. Is there any alteration in body cell mass prior to the onset of cachexia?

Previous studies have been limited to very sick patients or small numbers of patients. This study will attempt to further our understandig of the pathophysiology of covert cachexia before it becomes clinically evident. Patients and volunteers will be matched with respect to age, sex, and body surface area.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RS 10162-04 BEI

PERIOD COVERED: October 1, 1985 to September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.): Wound Healing: Biology and Rheology

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Thomas L. Talbot, MS, ACES, BEIB
Walter T. Lawrence M.D., C. Surgery, NCI
Lawrence E. Thibault, ScD. Bioengineering Dept, Univ. of Pa

COOPERATING UNITS (If any)

NCI, University of Pa, Philadelphia, Pa

LAB/BRANCH: Biomedical Engineering and Instrumentation

SECTION: Applied Clinical Engineering Section

INSTITUTE AND LOCATION: DRS, National Institutes of Health, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.2	1	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Preliminary studies have been completed with swine models. These studies involved stamping an indelible grid (10cmx10cm) on the skin of the swine, and the excision of a 1 cm by 10 cm strip of the skin out of the grid area, and finally approximating the incision edges with silk sutures. Photographs of the grid were taken before excision, after excision, and after suturing. These photographs will be analyzed to determine the impressed strain on the wound closure and eventually relate this information to wound breaking strength (WBS).

Studies based on a rat model that relate biologic and pharmacologic interventions to WBS have been completed. Certain groups were treated pharmacologically during the wound healing process. Significant decrease in WBS was observed in these groups as compared to control groups. Further studies will include the comparison of tumor bearing group to control groups.

Rats treated with 8 mg/kg Adriamycin prior to or on the day of wounding demonstrated decreased wound breaking strength in incisional wounds at all intervals after wounding. Decreased amounts of collagen and DNA, lower mitotic rates, and decreased cellularity were noted in wound chambers from rats treated in this manner. In both the incisional wound and wound chamber models, rats treated with Adriamycin seven days after wounding showed a less dramatic healing impairment. No differences in collagen type were noted between chambers from the Adriamycin treated and untreated rats. The data suggest that deficient quantities of platelet, macrophage, and lymphocyte factors; a decreased rate of collagen synthesis, and impaired collagen remodelling all contribute to the Adriamycin induced healing impairment.

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Wound healing was examined in two wound healing models in control rats and animals bearing methylcholanthrene induced sarcomas to evaluate experimentally the effects of tumors on wound breaking strength. In a dorsal incisional wound model, a significant decrease in wound breaking strength was noted from 19 days after the implantation of tumor onward. The size of the breaking strength deficit progressed with the size of the tumor. In a wound chamber model, hydroxyproline levels were measured as an index of collagen content, and 3 H-thymidine incorporation was related to DNA content to give an index of DNA synthesis. Histology and collagen types were also examined in the wound chamber model. The tumor bearing state produced no significant change in any of the parameters studied in the wound chambers. The tumor bearing state appears to inhibit wound healing in skin wounds but has no effect on healing in a model of a deeper wound. This difference in healing in the two wound models may be explained by a crosslinking deficiency or differential capacities for healing different types of wounds in tumor bearing rats.

PUBLICATIONS:

Lawrence, W., Norton, J., Harvey, A., Gorschboth, C., Talbot, T., Grotendorst, G.: Doxorubicin-induced impairment of wound healing in rats. JNCI. 76:1 119-126, 1986.

Lawrence, W., Norton, J., Harvey, A., Gorschboth, C., Talbot, T., Grotendorst, G.: Adriamycin induced impairment of wound healing. JNCI 1985.

Lawrence, W., Norton, J., Harvey, A., Gorschboth, C. Talbot T., Brotendorst, G.: Wound healing in sarcema benning rats: Tumor effects on cutaneous and deep wounds. J. Surg. Oncol 1985.

Lawrence, W., Norton, J., Harvey, A., Gorschboth, C., Talbot, T., Grotendorst, G.: Preoperative on Postoperative Adrianycin: Which is better for wound healing? Surgery 1986.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RS 10163-04 BEI

PERIOD COVERED: October 1, 1985 to September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.): Magnetoencephalographic Localization of Foci of Neurologic Activity

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	P.D. Smith	Physicist	BEIB	DRS
others	J.L. White	Electronic Eng.	BEIB	DRS
	W.S. Friauf	Chief	BEIB	DRS
	S. Sato	Neurologist	UCD	NINCDS
	D. Rose	Clinical Assoc.	MNB	NINCDS
	R. Porter	Chief	MNB	NINCDS

COOPERATING UNITS (if any): Medical Neurology Branch, NINCDS

LAB/BRANCH: Biomedical Engineering and Instrumentation Branch

SECTION: Electrical and Electronic Engineering Section

INSTITUTE AND LOCATION: DRS, National Institutes of Health, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

SQUID magnetometers have been used to study the magnetic activity associated with spike discharges occurring in interictal periods in patients with temporal lobe epilepsy. Magnetic field maps associated with specific spike discharges were obtained from measurements made with the SQUID at various locations on the skull. Mathematical models, which accounted for the head shape of the patient and the orientation of the SQUID, were developed to calculate the predicted magnetic field maps. These comparisons of the experimental and theoretical data indicate close agreement in some areas, but significant deviations in others, which are not explained by skull topography alone. A seven-channel SQUID has been obtained and is being evaluated.

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OBJECTIVE: To develop a non-invasive method for localizing neurological activity and, in particular, to determine the foci of spike discharges in temporal lobe epilepsy patients.

METHODS EMPLOYED: A single channel, second order magnetic field gradiometer has been used to study the MEG signals perpendicular to the surface of the scalp arising from spike discharges in patients with temporal lobe epilepsy. Simultaneous EEG scalp activity has been monitored using a full set of electrodes. Both MEG and EEG signals were recorded for typically 2 hours per session as the SQUID sensor was positioned at known locations across the surface of the scalp. A non-magnetic gantry and bed facilitated this positioning of the sensor with little inconvenience to the patient. Correlation of MEG and EEG recordings was performed, and based on these analyses magnetic field maps were generated for each specific type of spike discharge. A specially constructed device enabled accurate surface topography measurements to be made from a plaster cast replica of the patient's skull. A mathematical model was developed, based on Biot and Savart's law, which calculated the magnetic field gradient perpendicular to the surface of the skull at points corresponding to those measured with the SQUID. This model was encoded in Fortran and ran on an IBM-PC, which also had a surface contouring package available, to produce theoretical magnetic field distributions.

A seven-channel SQUID was acquired and installed in a gantry. Techniques for magnetically balancing the array of sensing coils from internal and external factors were evaluated. Environmental and instrumental noise measurements were performed. Preliminary measurements on several patients, including one with sub-dural electrodes in position, were performed.

MAJOR FINDINGS: In localizing the source of neurological activity associated with an epileptic spike discharge, the major limitation in producing a magnetic field map required to achieve this goal is the non-reproducible nature of the neurological event being measured. In fact, there are two aspects to this non-reproducibility; first, the signal derived from any given interictal discharge is variable due either to the recruitment of a variable number of neurons cooperating to produce the spike, or alternatively, to a change in orientation within a neural grouping. A second factor is that in many patients there exist multiple sites of neurological activity each of which elicit EEG and MEG signal. For these considerations, a multiple channel array has been installed to permit seven simultaneous position measurements to be made of the MEG signal associated with an individual spike discharge. The array comprises six 1.5cm diameter coils arranged in a circle with another single coil at the center; the spacing of the center coil to any of the coils in circle is 3cm, and the planes of the coils are arranged on the 10cm mean radius of the skull. Noise cancellation channels are provided. This detector is at the limit of current sensitivity and represents the maximum number of channels which present dewar technology can accommodate.

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Model analysis, based on the shape of the patient's skull and orientation of the SQUID with respect to it, explain in general the complex patterns obtained in the temporal region of patients with temporal lobe epilepsy. Excellent agreement is seen in the spherical regions of the skull, and the shape of the pattern in the temporal region is consistent with those observed; however, the magnitude of the extrema in this location is smaller than that calculated for the spherical region, a finding which is in direct contradiction to that observed. These findings are true for a variety of source-detector combinations. The source can be modelled as a point or an array of dipoles orientated at a chosen direction and the detector can be represented as either a point, area, or area gradiometer.

The most probable explanation for this discrepancy is the inadequacy of the "Biot and Savart model". In the current work, the volume currents flowing in the skull have been neglected, which is valid in spherical situations, but it appears from the map comparisons that they need to be included in regions differing significantly from sphericity.

Several installation problems have been overcome and the seven-channel SQUID is now operational. However, the present temporary location is magnetically noisy and the variable, unpredictable nature of this noise precludes any substantive measurements or evaluation of cancellation techniques to be made. The only practical solution for this environment would be to construct a shielded room. This may be desirable eventually, if the full sensitivity and capability of the SQUID is to be exploited. A new location, which has much less noise, will be available by Fall '86.

Facility with the operation of the instrument has been achieved and several patients have been scanned, including one with sub-dural electrodes in place. It was found these did not interfere significantly with the measurements, which were technically difficult to perform due to the large head bandage present. Correlation between MEG and EEG spikes in this patient was good, but little correlation between either the EEG or MEG and the multiple sub-dural recordings was observed. In the other patients, adequate signal to noise enabled the extrema to be located, but detailed mapping proved difficult.

SIGNIFICANCE: The potential of MEG to localize in three dimensions the source of neurological activity non-invasively offers a great advantage over conventional scalp EEG recording which suffers from the diffusion of scalp electrical potentials due to high skull resistance. The localization of epileptic foci in patients inadequately responding to drug therapy is important in the evaluation of surgical prospects and the direction of surgical procedures. The noninvasive localization of coordinates of normal and abnormal neurological activity in the brain offers the potential for greater understanding of the topography of human brain neurologic processing.

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PROPOSED COURSE: The mathematical model will be modified to account for the volume currents flowing in the head. Numerical integration techniques have successfully been applied to this problem in magnetocardiology, where the approximation to a sphere is not valid. These techniques appear appropriate for MEG. The seven-channel array, after installation in the new location, will be used to collect data for modelling and localization of foci. These data should provide insight on the non-stationary event nature of the inter-ictal discharges.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RS 10170-04 BEI

PERIOD COVERED: October 1, 1985 to September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Biological Applications of a Computer Controlled Analytical Electron Microscope

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

R.D. Leapman	Visiting Scientist	BEIB, DRS
Others:		
C.E. Fiori	Physical Scientist	BEIB, DRS
C.R. Swyt	Physical Scientist	BEIB, DRS
K.E. Gorlen	Electronic Engineer	CSL, DCRT
C.C. Gibson	Electronic Engineer	BEIB, DCRT

COOPERATING UNITS (If any)

CSLT, DCRT

LAB/BRANCH
Biomedical Engineering and Instrumentation Branch

SECTION
Electron Beam Imaging and Microspectroscopy Group

INSTITUTE AND LOCATION
DRS, National Institutes of Health, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Analytical methods have been developed and applied to electron energy loss spectra (EELS) and energy-dispersive X-ray spectra (EDXS) as well as their corresponding two-dimensional elemental maps produced by the computer-controlled analytical electron microscope in BEIB. Deconvolution algorithms developed earlier in the group have been applied to EELS spectra from cryosections of rapidly-frozen chromaffin cells in order to remove effects of plural inelastic scattering and to increase visibility of weak spectral features. In EELS elemental maps counting statistics are not high enough for a complete removal of plural inelastic scattering but sample-thickness effects can be corrected to first order by dividing the core loss signal by the zero-loss signal. Different algorithms for handling X-ray spectra have also been developed and comparison made in terms of accuracy and speed.

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OTHER INVESTIGATORS

A. Boyne — Dept. Pharmacology and Experimental Therapeutics, University of Maryland, Baltimore

A. Engel and R. Reichelt — Biozentrum, University of Basel, Switzerland

OBJECTIVES: To improve quantitation and detection limits for EELS and EDXS as applied to biological samples in the analytical electron microscope.

METHODS EMPLOYED: The Hitachi H700H analytical electron microscope operated under computer control has been developed further so that analytical methods can be applied to solve problems in cell physiology and cell biology. In addition to the Gatan 607 electron energy loss spectrometer extensive use is now being made of the Tracor Northern TN 5500 system which runs software for X-ray spectral analysis.

MAJOR FINDINGS: Plural scattering effects (both elastic and inelastic) can be successfully removed from EELS spectra and elemental maps. In the case of the elemental maps the correction procedure often leads to a contrast reversal in the image in thicker parts of the sample. In the analysis of energy dispersive X-ray specta the multiple least squares procedure generally compares favorably with the more sophisticated non-linear simplex fitting procedure. It has been demonstrated that quite low levels of calcium (< 0.3 mmole/Kg dry wt.) can be detected in the presence of potassium (overlapping K beta peak).

SIGNIFICANCE: The results from the insulin storage granules demonstrate that quantitative EELS data can be obtained from rapidly frozen secretory cells. This could lead to a better understanding of the mechanisms for insulin storage and release in the pancreatic beta cell. The apparent feasibility of low dose EELS phosphorus and nitrogen mapping in two dimensional membrane crystals is significant since it could provide a technique for localizing lipids and proteins in membranes without the limitations imposed by radiation damage.

PROPOSED COURSE: Thus far EDXS analysis is being sucessfully applied to a number of biological problems in the AEM. It is anticipated that the newer but promising techniques of EELS will also become as valuable after some improvements in instrumentation are incorporated over the next year or two. A multichannel parallel detection system capable of improving EELS sensitivity by as much as a factor of 1000 is currently on order. In addition a dedicated field emission STEM with better than 0.5 nm resolution is expected to be delivered in one year.

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PUBLICATIONS

Leapman, R.D.: Quantitative electron energy loss spectroscopy and elemental mapping in biology. Microbeam Analysis, (in press) San Francisco Press, 1986.

Leapman, R.D.: STEM elemental mapping by electron energy loss spectroscopy, Annals of N.Y. Acad. Sci (in press) 1986.

Leapman, R.D.: Electron energy loss spectroscopy, in: Electron Microscopy in Medical Research and Diagnosis: Present and Future Directions, Editor J.E. Johnson, Jr., Journal of Electron Microscopy Technique (Alan Liss Publishers) Vol 4, 95-102, 1986.

Livne, E., Oliver, C., Leapman, R.D., Poole, A.R.: Age-related changes in the role of matrix vesicles in the mandibular condylar cartilage, J. Anatomy London (in press).

Silbermann, M., Tenenbaum, H., Livne, E., Leapman, R.D., von der Mark, K., Hari Reddi, A.: In Vitro transformation of chondroprogeritor cells into osteoblasts and the formation of a new chondroid bone in a serum-free hormone-supplemented medium. Calcified Tissue International (in press).

Reichelt, R., Engel A., Leapman, R.D.: Low dose electron energy loss spectroscopy by scanning transmission electron microscopy. In: Proc. X1 International Congress on Electron Microscopy, Kyoto, Japan (in press) 1986.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RS 10184-03 BEI

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Physical Chemistry of Biological Macromolecules

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Marc S. Lewis	Research Chemist	BEIB/DRS
S.I. Chung	Research Chemist	LB NIDR
C.B. Klee	Section Chief	LB NCI
R.E. Lippoldt	HSO Dir., USPHS	CEB NIDDK
S.W. Luborsky	Research Chemist	DCBD NCI
K. Prasad	Visiting Fellow	CEB NIDDK
R.J. Youle	Research Chemist	SNB NINCDS

COOPERATING UNITS (if any)
LB/NIDR, LBDA/NIDR, SNB/NINCDS

LAB/BRANCH
Biomedical Engineering and Instrumentation

SECTION
Analytical Methods

INSTITUTE AND LOCATION
DRS, National Institutes of Health, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.9	0.9	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this project is to study the physical properties of a wide variety of biological macromolecules with the goal of correlating these properties to the structure and function of the macromolecules. The emphasis is on the thermodynamics of the interactions of these macromolecules and on their molecular size and shape. Analytical ultracentrifugation and mathematical modeling are the principal research techniques used.

Studies on the association of fibrinogen with other proteins involved in blood clotting and fibrinolysis have been continued and currently deal with the association of fibrinogen with plasma and platelet Factor XIII and with the association of plasminogen with the D and E fragments of fibrinogen.

Research on ricin has involved studies on a monoclonal antibody to the ricin B chain, the interaction of this antibody with ricin, and its effect on ricin toxicity both in vitro and in vivo. Research on the 27 S intermediate form of clathrin involved studies on the mechanism of formation of this form of clathrin and its relationship to the formation of clathrin baskets.

Studies on synapsin have been directed toward determining the molecular weight of the monomeric form of this protein and determining whether the aggregation which it undergoes is reversible, irreversible or a combination of both. Studies on cell adhesion peptides have been directed toward determining their mode of self-association, measuring the thermodynamic parameters characterizing their self-association, examining their possible interactions with each other and attempting to establish a relationship between their associative behavior and their primary structure.

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METHODS EMPLOYED: The macromolecules have been isolated and purified from appropriate sources by conventional methods. The associations have been studied by establishing equilibrium gradients in the analytical ultracentrifuge and then analyzing the resultant data by means of mathematical modeling techniques using the MLAB system operating on the DEC-10 computer.

MAJOR FINDINGS: Proteins Involved in Blood Clotting: Studies on the association of both plasma and platelet Factor XIII with fibrinogen show that, like the association of plasminogen with this protein, there are four binding sites per fibrinogen molecule, that the binding is so highly cooperative that the presence of complexes of Factor XIII with fibrinogen involving the binding of fewer than four molecules of this protein per molecule of fibrinogen can not be effectively demonstrated, and that the total free energy changes for the binding of the four moles of either platelet or plasma Factor XIII are respectively -30 and -36 kcal per mole of fibrinogen.

Ricin: It was demonstrated that a monoclonal antibody to purified ricin B chain reduced ricin toxicity 30-100 fold in vitro by blocking ricin binding to cell surface galactose-containing receptors. Ultracentrifugal analysis demonstrated a 1:1 stoichiometry of ricin and antibody and an association constant of 8.8×10^6 per mole. This association was reduced 5 fold by 20 mM lactose and 15 fold by 100 mM lactose but was not reduced at all by sucrose in either concentration. Like lactose, the antibody blocked the non-target toxicity of ricin immunotoxins in vitro. In vivo, unlike lactose, the antibody protected mice from ricin immunotoxin toxicity.

Clathrin: The 27 S intermediate obtained by dialyzing clathrin against 2 mM MES buffer at pH 5.9 was demonstrated to be a reversible monomer-hexamer association with a free energy change of -44.7 kcal per mole of hexamer formed. It was also demonstrated that the clathrin baskets formed from the 27 S imtermediate exhibited a much greater degree of homogeneity of size distribution than did baskets which were directly formed and did not pass through the 27 S intermediate size.

Synapsin: Synapsin I is a protein isolated from brain tissue and appears to be associated with synaptic vesicles. Ultracentrifugal studies demonstrated a momoner molecular weight of 62000 and that this protein showed a marked tendancy to undergo aggregation to form dimers, tetramers, octamers and possibly higher aggregates. What has yet to be established is whether this aggregation is reversible, irreversible or a combination of both.

Cell adhesion Peptides: The cell adhesion peptides under investigation here are small peptides of from four to ten amino acids whose sequence is derived from the sequence of fibronectin. They have been isolated from enzyme digests of purified fibronectin or by synthesis and have been purified by HPLC. Ultracentrifugal analysis shows that all of the peptides studied reversibly self-associate to form dimers with a free energy change in the range of -0.5 to -1.5 kcal per mole, with the free energy of association depending on the amino acid sequence of the peptide.

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SIGNIFICANCE: The studies on the interactions of fibrinogen and other proteins such as plasma and platelet Factor XIII are relevant to the very significant roles that these proteins play in blood clotting and subsequent fibrinolysis. It is our objective to correlate the physical chemistry of these interactions with the physiological role which these proteins play. The studies on ricin are relevant to an understanding of the mechanisms of ricin and ricin immunotoxin toxicity and may be useful in the development of the application of ricin immunotoxins in cancer chemotherapy. The studies on clathrin are relevant to an understanding of the mechanisms involved in the formation of clathrin baskets and coated vesicles. The studies on synapsin are relevant to the role this protein might play in synaptic vesicles and their possible role in neural transmission. The studies of cell adhesion peptides are of importance for two different reasons. They are of possible relevance to an understanding of the role of fibronectin in cell adhesion leading to the formation of tissues and organs and in adhering to artificial substrates. They also offer the possibility of new knowledge leading to an understanding of the role of primary amino acid sequence in the mechanisms involved in the interactions of peptides and proteins.

PROPOSED COURSE: The studies on the binding of plasma and platelet Factor XIII by fibrinogen were presented at the annual meeting of the American Society of Biological Chemists and a manuscript on this work is currently in preparation. More studies on the interactions of the proteins involved in blood clotting and subsequent fibrinolysis will be pursued extensively, as this appears to be a very fruitful area of endeavor.

A manuscript describing the ricin studies has been submitted to the Journal of Immunology. We plan to continue work in this area with further studies on the relationship of the thermodynamics of ricin subunit association to the mechanism of the reaction, with studies on the association properties of other related A and B chain type toxins and with studies on the interaction of ricin with specific antibodies.

A manuscript on the studies on clathrin described above is currently in press. Because of the death of Dr. Edelhoch it is not possible at this time to predict what further collaborative work will be done on clathrin after some further studies on the formation of the 27 S intermediate now progress have been completed.

Studies on synapsin will be continued in order to determine the nature of the mechanism or mechanisms involved in the aggregation of this protein.

Further studies on the cell adhesion peptides will be directed toward obtaining the values of the changes of entropy, enthalpy and specific heat capacity characterizing their self-association with the intention of attempting to relate the values of these thermodynamic parameters to the mechanisms involved in the self-association and to the primary amino acid sequence. The possible association of these peptides with each other will also be studied.

PUBLICATIONS

Parasad, K., Lippoldt, R.E., Edelhoch, H., Lewis, M.S.: An intermediate polymer in the assembly of clathrin baskets. Biochemistry (in press).

Lewis, M.S., Youle, R.J.: Ricin subunit association; thermodynamics and the role of the disulfide bond in toxicity. Journal of Biological Chemistry (in press).

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01RS 10185-03BEI

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Interventional Catheter Development

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

John W. Boretos, Physical Scientist, BEIB, DRS

Others:

John Doppman, M.D.	Radiologist	CR, CC, NIH
Edward Oldfield, M.D.	Neurosurgeon	NINCDS, NIH
Robert L. Dedrick, Ph.D.	Chem. Engr.	BEIB, DRS, NIH

COOPERATING UNITS (if any)
CR, CC, NIH; NS, NINCDS, NIH,
BEIB, DRS, NIH

LAB/BRANCH
Biomedical Engineering and Instrumentation

SECTION
Chemical Engineering Section

INSTITUTE AND LOCATION
DRS, National Institutes of Health, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.4	0.7	0.7

CHECK APPROPRIATE BOX(ES)

- [X] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of the project is to develop a catheter system for interventional radiology which is capable of traversing small and branching blood vessels (i.e. 1.5 to 4.0 mm) to reach the proximity of tumors and to administer chemotherapeutic drugs to these tumors in a controlled manner. A major obstacle to effective treatment is thought to be maldistribution of the drug at the site due to inadequate mixing with the blood. A multi-lumen catheter, which is propelled and directed through these small blood vessels via jets of fluid emanating from its distal end, continues to show promise for negotiating heretofore difficult to reach areas. Fluid turbulence generated by drug emanating at retrograde angles from the tip of the catheter adds significantly to the mixing of the drug within the blood stream. Animal tests have substantiated the feasibility of the catheter system for clinical use. A transparent model of the vascular network simulating blood flow of a pulsatile nature was designed to evaluate the efficiency of the jet flow at various delivering rates. A high torque roller pump using custom designed polyurethane tubing is capable of producing satisfactory infusion rates over an 8 hour period.

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OBJECTIVES: The objective of this project is to develop a catheter system for interventional radiology which is capable of traversing small and branching blood vessels (i.e., 1.5 to 4.0 mm) to reach the proximity of cancerous tumors and to administer chemo-therapeutic drugs to these tumors in a controlled manner.

METHODS EMPLOYED: A multi-lumen microcatheter which is propelled and directed through small blood vessels via jets of fluid emanating from its distal end has shown promise for negotiating heretofore difficult to reach areas. The catheter is controlled by an adjustable pressurized manifold operated by foot switches. Radiopaque contrast solution is used to propel and visualize the movement of the catheter by being pumped selectively through the lumens of the catheter. As radiopaque fluid jets out of the catheter ports, thrust is transmitted to the tip of the catheter. Right, left, and forward motions can be controlled in this manner. The chemotherapeutic drug is brought on-line by valvular switching at the manifold to replace the contrast agent. The jet action of the fluid emanating at a retrograde angle from the tip of the catheter generates fluid turbulence which adds significantly to the mixing of the drug within the blood stream. A transparent model of the vascular network simulating pulsatile blood flow is being used to evaluate the efficiency of the jet catheter system and its behavior in administering chemotherapeutic drugs. Single lumen catheters capable of effective mixing where maneuverability is not a demanding requirement are being developed for liver tumor treatment.

MAJOR FINDINGS: The multi-lumen catheter which is propelled and directed through small blood vessels via jets of fluid emanating from its distal end continues to show promise for negotiating heretofore difficult to reach areas of the vascular network. The jet propulsion action can be regulated to serve as a means of administering chemotherapeutic drugs. Because of its angular retrograde flow, mixing of the drug within the blood stream is significantly enhanced over that of an open ended catheter of a single orifice. Custom designed polyurethane tubing used with a high torque roller pump can provide constant perfusion of drug through the catheter system at back pressures of 60 psi for up to 8 hours. Metallic wire coils are being developed to act as the catheter body where high pressure is to be encountered.

SIGNIFICANCE: Further development of the catheter system is planned to increase fluid volumes under lower induced pressures along with characterization of the efficiency of intravascular drug administration. Clinical use is underway.

PUBLICATIONS

Boretos, J.W., Girton, M.E. and Doppman, J.L.: Polyurethane radiopague marker for indwelling devices. J. Biomed. Mater. Res., 20. 273-275, 1986.

Lutz, R.J., Dedrick, R.L., Boretos, J.W., Oldfield, E.H., Blacklock, J.B., Doppman, J.L.: Mixing studies during intracarotid artery infusions in an *in vitro* model. J. Neurosurg.64: 277-283, 1986.

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Boretos, J. W., Sullivan, J. V., Fitze, P. E., Girton, M. E., and Doppman, J. L. "Jet directed catheter for interventional radiology, Annals of Biomedical Engineering. 14: 323-338, 1986.

Doppman, J. L., Dedrick, R. L., Shook, D. R., Lutz, R. J., Goldstein, S. R., Blacklock, J.B., Boretos, J. W., Paul, R. H., Austin, H. A. III, Bowman, R. L., and Oldfield, E. H.: Glioblastoma: catheter techniques for isolated chemotherapy perfusion. Radiology,159: 477-483, 1986.

Boretos, J. W., Lutz, R. J., Dedrick, R. L., and Doppman, J. L., : Retrojet catheter for intra-arterial infusion. (Abstract) 39th Proceedings Alliance for Engineering in Medicine and Biology. Sept 13-16, 1986.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RS 10187-03 BEI

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Mixing Studies during Infusion via Catheter

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

R.J. Lutz	DRS, BEIB	J. Boretos	DRS BEIB
R.L. Dedrick	DRS, BEIB	M. Bretz	DRS, BEIB
E. Oldfield	NINCDS, IR, SN	C. McClure	DRS, BEIB
J. Doppman	CC, DR		
D. Miller	CC, DR		
J. Collins	CC, DR		

COOPERATING UNITS (if any)
NINCDS
CC

LAB/BRANCH
Biomedical Engineering and Instrumentation

SECTION
Chemical Engineering Section

INSTITUTE AND LOCATION
DRS, National Institutes of Health, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Intravascular catheterization is a very common method of drug adminstration. Proper utilization of catheters requires knowledge of the physio-chemistry of the drugs in solution, the relevant pharmacokinetics of drug disposition, and in some instances the fluid mechanical events that occur as solutions are infused into the blood stream. For example, intra-arterial chemotherapy offers an alternative to systemic (iv) chemotherapy for localized neoplasms. Infusion of chemotherapeutic agents directly into the arterial supply of a tumor can allow high local drug concentrations in the tumor-bearing tissue while minimizing systemic toxicity. Application of this procedure in patients has produced varied results. For example, intracarotid infusions of cisplatin and BCNU have shown some evidence of regression of glioblastomas, but have also resulted in frequent episodes of leukoencephalopathies and other focal lesions, such as ophthalmic toxicity. These findings suggest the influence of streaming of the drug solutions from the catheter tip and inadequate mixing and distribution to distal tissue regions. This hypothesis was verified in an in-vitro flow model of the human carotid artery system. The results of dye injection studies indicated that streaming is likely to occur during low infusion rates, and that mixing can be enhanced by increasing infusion velocities, by improving catheter tip design, and also by modifying the mode of injection such as infusing transverse or retrograde to the blood flow.

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In vivo studies involving both slow and fast infusions of radiolabeled iodoantipyrine into carotid arteries of monkeys have corroborated the findings of the *in vitro* model studies. Similar streaming effects were observed during *in vitro* infusion studies in a glass model of the hepatic artery system. The practice of using multilumenal catheters for the simultaneous infusion of incompatible drugs is being investigated from a fluid-mechanical point of view to ascertain if adverse interactions can occur among the several drug solutions. Unwanted drug interactions such as precipitation or inactivation can be detrimental to patients.

OBJECTIVES: To assess the degree of mixing produced by infusion of solutions through various types of catheters in *in vitro* models of the carotid and hepatic artery systems.

To determine methods to enhance mixing, such as increasing infusion velocities and improving catheter tip design.

To study other mixing phenomena in intravascular catheter systems such as simultaneous infusion of incompatible drugs through multilumen catheters.

METHODS EMPLOYED: A clear plastic model of the human internal carotid artery system and several major branches was fabricated by casting a resin (Alplex) around a low-melting point alloy (Cerroloy) which was sculpted into a geometry determined from human angiographic photographs. A glass model of a portion of a human hepatic artery system was also constructed according to a sequence of angiographic pictures. Each model was connected to its own circulatory flow loop which produced steady or pulsatile flow through the models. Still photographs, 16mm movie films, and video recordings were made of dye injections into a flowing blood stimulant (40% glycerine/water). A range of infusion rates was studied using several types of catheters positioned in numerous locations within the lumen of the flow model.

Fluid samples were taken from the various branch arteries downstream, and dye concentrations were analyzed spectrophotometrically to arrive at a quantitative measure of dye distribution distal to the point of infusion. This data was represented in the form of histograms of branch dye concentration relative to a well mixed concentration. The carotid model has six collection sites; the hepatic model has 16 sites.

A very preliminary rigid glass model of the vena cava system was fabricated to observe the infusion pattern of dye solutions from a number of multilumen catheters (eg Hickman, and Arrow-Howes). Movie photographs (16mm) of dye injections were made to record the patterns of infusion at 2 ml/minute. Glycerine/water solution from a pulsatile flow system simulated blood flow.

MAJOR FINDINGS: Streaming of dye solutions in the carotid model has been demonstrated to occur from several types of catheters in both the carotid and hepatic models when infusion velocities are slow relative to the surrounding fluid velocities. Mixing can be enhanced by increasing the infusion rate.

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Catheters that eject solutions retrograde to the blood flow show greater degrees of mixing at a given infusion rate. In the hepatic flow model, infusions that were done with the catheter tip retracted into the side arm of the gastroduodenal artery remained in a boundary layer close to the wall of the proper hepatic, and preferentially exited out one branch (the left hepatic). End hole catheters that were extended into the main stream of the common hepatic flow showed very distinct streamlines of dye from their tip. Infusion rates of 20 ml/minute or greater were required to give significant mixing. Transverse ejection of dye from modified catheter tips (jet) gave enhanced mixing. As with carotid model studies, concentration histograms were sensitive to catheter position. Previous in vivo experiments using radiotracer infusions into monkey carotid arteries corroborated the in vitro model studies.

Preliminary experiments with simultaneous infusion of dye solutions through multilumen catheters would indicate that mixing of solutions is quite good and very rapid, since these studies simulated high volumetric flow regions in the vena cava. Dilution of infusion solutions with blood probably occurs quickly.

SIGNIFICANCE: Inadequate mixing of drugs infused via intra-arterial catheters can result in variable drug exposure to distal tissue regions. Excessive drug exposure, particularly to sensitive normal tissue such as the eye, can result in unwanted toxic effects. Inadequate drug exposure can result in reduced therapeutic effect at a particular tumor site. With inadequate mixing, the exposure at all sites would be random and unpredictable.

PROPOSED COURSE: To continue seeking methods for improving mixing during intra-arterial infusion, looking at different modes of injection, improved catheter tip design, and optimum catheter placement.

To replace glycerine/water and test the in vitro flow system and model with a new polymer blood simulant that has non-Newtonian properties.

To conduct further studies on infusion of incompatible drug solutions through multilumen catheters using an in vitro model.

PUBLICATIONS

Lutz, R.J., Dedrick, R.L., Boretos, J.W., Oldfield, E.H., Blacklock, J.B., and Doppman, J.L.: Mixing studies during intracarotid artery infusions in an in vitro model. J. Neurosurg. 64: 277-283, 1986.

Blacklock, J.B., Wright, D.C., Dedrick, R.L., Blasberg, R.G., Lutz, R.J., Doppman, J.L., and Oldfield, E.H.: Drug streaming during intra-arterial chemotherapy. J. Neurosurg. 64: 284-291, 1986.

Doppman, J.L., Dedrick, R.L., Shook, D.R., Lutz, R.J., Goldstein, S.R., Blacklock, J.B., Boretos, J.W., Paul, R.H., Austin, H.A., Bowman, R.L., and Oldfield, E.H.: Glioblastoma: catheter techniques for isolated chemotherapy perfusion. Radiology, 159: 477-483, 1986.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RS 10191-03 BEI

PERIOD COVERED: October 1, 1985 to September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.): Continuous Optical Monitoring for Bacterial Cultures

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

G.M. Maxwell	Staff Fellow	BEIB, DRS
S.B. Leighton	Senior Engineer	BEIB, DRS
Ernst Freese	Chief	LMB, NINCDS
S.A. Robrish	Scientist	LMI, NIDR
Y. Shiloch	Chief, Pilot Plant	LCDB, NIADDK

COOPERATING UNITS (If any)
LMB, NINCDS
LMI, NIDR
LCDB, NIADDK

LAB/BRANCH: Biomedical Engineering and Instrumentation Branch

SECTION: Mechanical Engineering Section,

INSTITUTE AND LOCATION: DRS, National Institutes of Health, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.3	.3	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The incubation of bacterial cultures is a standard laboratory technique in numerous biological studies. Monitoring the growth of these cultures in a shaker bath or fermenter currently requires frequent manual sampling. The work on this project has been aimed at developing a system capable of continuously monitoring growth in situ using optical measurement techniques.

To date the work has demonstrated the feasibility of making these measurements in shaker flasks employing an immersed detection system and yielded a prototype system for this application. Additional efforts have been directed at designing a system which will operate in a commercial type fermenter. The principal problem of eliminating gas bubbles in the optical path of the immersed probe has been solved.

The shaker flask probe consist of a light emitting diode and a photodetector mounted a right angles to one another in a fixed submersible head. Readings are taken at intervals of one minute under control of a computer system which also collects and process data.

The fermenter probe is of a similar design except that the optics are kept in a bubble free region of the housing.

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OBJECTIVES: The objectives of this project are twofold 1) To develop a system capable of continuously monitoring the growth of bacterial cultures in shaker flasks 2) To develop a system capable of monitoring the growth of microbial cultures in a fermenter.

METHODS EMPLOYED: The probe consists of a sensor head which holds a red light emitting diode (LED) producing a narrow beam at right angles to a photodiode. A baffle is built into the sensor head so that transmitted light does not strike the photodiode. The sensor head is potted in silicone rubber to isolate the diode and the wiring electrically from the conductive culture media. Attached to the sensor head is a connecting rod and a spacing washer which serve to keep the sensing head immersed and to maintain the alignment of the device in the shaker flask. A hole through the spacing washer provides an access port for adding or withdrawing solution from the culture with a pipette. Both the LED and the photodiode are connected by a shielded and insulated wire to the external electronics package. Contained in the electronics package are a current-to-voltage converter and filter for the photodiode output and a driver circuit for the LED. The sampling of the output voltage, the state of the LED, and the control of the shaker bath are managed by a personal computer which also maintains and analyzes the data. The present version of the probe can be sterilized with ethylene oxide

A data collection cycle requires stopping the shaker bath to permit gas bubbles in the solution to rise out of the light path of the detector; in aerated cultures ths period was typically two seconds but it may be varied under program control to accommodate aerated solutions that clear at a faster or slower rate. The output of the detector is sampled both with and without the LED turned on to permit correction for ambient light conditions.

The probe's performance was evaluated by comparison with the readings obtained with a spectrophotometer on samples periodically withdrawn from the growing culture. Monitored were the growth of Bacillus subtilis, Escherichia coli, and Saccharomyces cerevisiae.

Calibration of the probe was made by using the spectrophotometer values to determine the coefficients A and B for the equation.

$$OD = A \times (V_x - V_0)$$

where OD is measured by the spectrophotometer, V_x is the output voltage of the current-to-voltage converter, and V_0 is the output voltage measured in the growth medium prior to inoculation.

The design has been optimized for the particular micobes and growth conditions typically employed in this laboratory. For example, B. subtilis undergoes six to seven doublings after inoculation in a typical experiment which corresponds to a change of approximately 3.0×10^7 to 2.1×10^9 colony forming units per millilter.

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MAJOR FINDINGS: A multi-channel system for shaker flask monitoring has been completed. This device is capable of monitoring the growth of microbes in the density range of 3.0 x 10 to 2.1 x 10^9 colony forming units per milliliter of Bacillus subtilis. To date this device has been used to monitor the growth of Bacillus subtilis, Escherichia coli, and Saccharomyces cerevisiae.

A protype system has been developed for use in a fermenter which presently consists of the immersible head of the shaker flask design housed in a special device which creates a bubble free region within the fermenter. Tests with the anaerobe Fusobacterium nucleatum have yielded results with a correlation of 0.99 and a mean error of 5% compared to colorimetric measurements.

For these particular microbes, adhesion of microbes to the surface of the detectors has not been a problem over the time course of a typical experiment at the concentration levels obtained for the media employed.

SIGNIFICANCE: A shaker flask system with continuous monitoring has several advantages over manual sampling: 1) it is a labor saving device and as such frees the experimenter from taking manual samples, therefore permitting a larger number of cultures to be run simultaneously 2) it decreases the risk of contamination as the flask need not be opened for sample removal 3) it removes fluctuation in culture volume caused by manual sampling as a variable 4) it permits close monitoring of cell cultures with a rapid growth rate.

The fermenter control system will permit the continuous measurement of microbial population density in an automated fashion. This is of particular utility since many fermentation growths extend over a period of days. Additionally, the fermenter monitor creates the possibility of maintaining a constant cell density within the fermenter which would permit long time course experiments under constant culture conditions and would aid in the harvesting of culture products.

PROPOSED COURSE: Future work on the fermenter monitoring system will be aimed at evaluating techniques for handling the problem of cell growth on detector surfaces and at determining appropriate materials and construction techniques for an autoclavable design.

PUBLICATIONS:

Maxwell, G.M. Robrish, S.A. "Continuous Optical Monitoring of Microbial Concentrations," Proceedings of the First Annual Conference on Biotechnology, ASM, March, 1986.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RS 10193-03 BEI

PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Development of Everting Toposcopic Catheter for Clinical Gastroenterological Use

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

D.R. Shook, Biomedical Engineer, BEIB,DRS

COOPERATING UNITS (If any)

Georgetown University Medical Center (E.L. Cattau); Esophageal, Gastric and Colonic Diseases, Digestive Diseases and Nutrition Division, NIADDKD (K.J. Vener).

LAB/BRANCH

Biomedical Engineering and Instrumentation Branch

SECTION

Mechanical Engineering Section.

INSTITUTE AND LOCATION

DRS, National Institutes of Health, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	

CHECK APPROPRIATE BOX(ES)

- [X] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The unique characteristics of the everting catheter have suggested its application in the gastrointestinal system. The operation of the catheter promises to facilitate the catheterization of the pancreatobiliary ducts through the Papilla of Vater. An initial series of patients has undergone routine endoscopic retrograde cholangiopancreatography (ERCP) using the catheter for contrast infusion. An extensively modified catheter is passed through an appropriately positioned endoscope. The catheter tip is placed within the ampulla, and the element is everted. In basic ERCP, contrast medium is then injected for diagnostic procedures. Catheter modifications include an overall lengthening to approximately 200 cm, while eliminating any added length to the everting element beyond that required by the clinical procedure. This keeps frictional drag of the element to a minimum. Where kinking of the tip of the primary catheter in the elevator of the scope presents a problem, reinforcement of the tip or a change to PTFE materials is made. In addition, the catheter's ability to atraumatically negotiate small, tortuous ducts is being investigated for catheterization of remote pancreatic and cystic ducts for sampling of pure secretions, placement of biliary stents, and conveyance of CCD television cameras.

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OBJECTIVES: Develop techniques for selective catheterization of small, tortuous ducts of the gastrointestinal system and optimize apparatus for varied diagnostic and therapeutic applications.

METHODS: The catheters are handmade to suit individual procedures. Polyurethane materials are used, in general, but PTFE is often required for the primary catheter to allow full tip manipulation. In this case, the PTFE is chemically etched to permit cohesive bonding to the polyurethane everting element. Lengths of the various components of the assembly are customized at fabrication; the overall design of the everting catheter facilitates these changes.

MAJOR FINDINGS: Experimental work has progressed sufficiently to justify catheterization of patients during routine ERCP procedures. A patient series has been started using a catheter modified for insertion and operation through a fiberoptic endoscope. The far reaches of the pancreatic duct have been safely catheterized and opacified at lower than conventional pressure. The five patient series has indicated that a more easily manipulated primary catheter material is required for convenient, full range ERCP techniques. The everting catheter is being adapted, therefore, by changing the primary catheter material to polytetrafluoroethylene (Teflon) and developing a technique to bond the polyurethane everting element to that material. Initial efforts have been encouraging, and another patient series for verification is underway.

SIGNIFICANCE: Catheterization of the far reaches of the pancreatic duct and branches coupled with sampling of pure secretions will reveal heretofore unavailable data on secretory activities as functions of digestion time and pathological state. Also of interest to clinical research is conveying fiberoptic probes for measuring chemical activity levels as near to the secretory source as possible. The catheter may greatly facilitate these applications without mechanical trauma and with much less risk of pancreatitis. The catheter has the potential to repeatedly and atraumatically negotiate the cystic duct for sampling of fluids and possible infusion of chemotherapy for dissolution of gall stones. The conveyance of a 3F CCD camera will allow visualization of cholangiopancreatic lesions.

PROPOSED COURSE: Optimize catheter system for diverse clinical applications. Substantiate the use of the everting concept in unique GI applications. Develop related devices and explore additional uses for the catheter.

Related Project Numbers

Z01-RS-10015-11 BEIB (Vascular use)
Z01-RS-10194-03 BEIB (Polymer Processing)

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RS 10194-03 BEI

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Optimized Polymer Processing for Advanced Catheter Development

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

D.R. Shook, Biomedical Engineer, BEIB, DRS

COOPERATING UNITS (if any)
Diagnostic Radiology, CC (J.L. Doppman); Esophogeal, Gastric and Colonic Diseases, Digestive Diseases and Nutrition Division, NIADDKD (K.S. Vener)

LAB/BRANCH
Biomedical Engineering and Instrumentation Branch

SECTION
Mechanical Engineering Section

INSTITUTE AND LOCATION
National Institutes of Health, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.05	0.05	

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects
  - ☐ (a1) Minors
  - ☐ (a2) Interviews
- ☐ (b) Human tissues
- ☒ (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Advanced techniques of plasticating extrusion can produce materials of optimized anisotropic properties and very fine dimensional characteristics. Availability of such materials then supports development of unique polymeric medical devices, such as the everting catheter. Unique polymer resins are first characterized specifically for extrusion characteristics, and predictive models of rheological behavior are constructed. Die designs can now be implemented to take advantage of unique polymer character and to overcome the most critical processing difficulties. The models may also be used to eliminate certain polymers from consideration. Current efforts are to optimize the catheter material for maximally safe operation and to allow development for arterial dilatation.

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OBJECTIVES: To produce advanced and state-of-the-art extrusions to allow development of unique catheter and general medical devices for diagnostic and therapeutic applications.

METHODS: A 0.75 inch plasticating extruder has been extensively modified for the high precision processing of polyurethames. Temperature and pressure control zones allow full control of melt rheology to produce extremely thin-wall tubing. The annular die allows pressure controlled air sizing and the peripheral equipment can accommodate a broad range of melt temperatures and line speeds.

MAJOR FINDINGS: The processing system has been assembled and optimized for fine wall tubing extrusion. Major problems with the pressure and temperature control systems have been corrected. The rheological model of the everting catheter polyurethane is being developed. Phase I of that development, verification of the proposed model by analysis of a known material, is complete. Large time lags between temperature control and measurement and large swings in temperature caused lack of correlation between model and data. Therefore, an additional control zone is being added to the extruder to more closely control those variations.

SIGNIFICANCE: The availability of advanced materials processing technology is often the critical element in development of unique polymeric medical devices. The everting catheter is an excellent example of a very useful device whose development was substantially delayed by lack of high quality processing capabilities. The economics of the medical device industry often restrict development efforts to known low-risk technologies. The present processing capabilities will permit and encourage the development of unique device designs that will reveal clinical research applications not heretofore envisioned.

PROPOSED COURSE: To optimize everting catheter materials for maximum safety; to broaden catheter applications toinclude arterial dilatation by customizing material anisotropies; to implement design modifications to allow convenient and reliable steering; broaden the available size range of the catheter to include 2F for fallopian tube use.

RELATED PROJECT NUMBERS

Z01-RS-10015-11 BEIB (Vascular use)
Z01-RS-10193-03 BEIB (GI use)
Z01-RS-10226-01 BEIB (Fallopian tube Use)

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RS 10201-02 BEI

PERIOD COVERED: October 1, 1985 to September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.): Automated Cell Colony Scanner System

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Dr. Seth Goldstein, Chief, Mechanical Engineering Section, BEIB, DRS
Dr. Michael Brownstein, Chief, Laboratory of Cell Biology, NIMH

COOPERATING UNITS (if any)

Laboratory of Cell Biology, NIMH

LAB/BRANCH: Biomedical Engineering and Instrumentation

SECTION: Mechanical Engineering Section

INSTITUTE AND LOCATION: DRS, National Institutes of Health, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	.7	.3

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects
  - ☐ (a1) Minors
  - ☐ (a2) Interviews
- ☐ (b) Human tissues
- ☒ (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A system is being developed for the purpose of identifying, for subsequent cloning, a minute fraction of cells possessing a specific antibody or other fluorescent tag. Major emphasis will be put on making the system as simple and inexpensive and readily available to investigators as possible. A variety of concepts and techniques were identified and evaluated, and the most promising has been developed into a computer controlled instrument.

BEST COPY AVAILABLE

OBJECTIVES: To develop a relatively inexpensive system to identify a minute fraction of cells having specific antibodies so they can subsequently be cloned.

SIGNIFICANCE: Many studies involving the "shotgun" approach to genetic engineering could be performed far more efficiently and easily if it were possible to identify the small percentage (e.g., 10^{-4}%) of cells that possess a particular antibody, using a relatively simple and inexpensive system. Whereas in principal, this is possible to do with a laser activated cell sorter, these devices are quite expensive and are therefore not always readily available to investigators, and in some cases are not appropriate for a variety of reasons. It is anticipated that development of a system which overcomes these disadvantages should be of widespread interest to investigators using genetic engineering techniques and monoclonal antibodies.

METHODS: The system configuration selected uses a commercially available x-y recorder to move a tissue culture dish in a raster pattern thru a stationary laser beam located directly over a photomultiplier tube. Front end electronics processes the PMT output which, when appropriate, is fed into an IBM PC along with current coordinant information. A localized fine scan is performed under computer control, and if an artifact check is negative, the precise location of the fluorescent colony is scribed on the bottom of the dish. Subsequently, in a separate procedure colonies adherent to the dish above the scribe mark are removed and cloned.

FINDINGS: The original system worked well on bench tests with artificial targets but was prone to fluorescent artifacts caused by cell debris. An improved front end of the system to overcome this problem has been successfully bench tested on a variety of artificial targets and will be tested with cell culture dishes shortly.

PROPOSED COURSE: Evaluation of the prototype system will continue using tissue culture dishes with test targets and cell cultures. System parameters will be optimized to obtain the best compromise between sensitivity and artifact rejection.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RS 10202-02 BEI

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Optical Techniques for Dental Instrumentation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)
G.M. Maxwell, Senior Staff Fellow, BEIB, DRS
R.L. Webber, Chief, CIB, NIDR

COOPERATING UNITS (if any)
CIB, NIDR

LAB/BRANCH
Biomedical Engineering and Instrumentation Branch

SECTION
Mechanical Engineering Section

INSTITUTE AND LOCATION
DRS, National Institutes of Health, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.3	.3	

CHECK APPROPRIATE BOX(ES)
- ☐ (a) Human subjects
  - ☐ (a1) Minors
  - ☐ (a2) Interviews
- ☐ (b) Human tissues
- ☒ (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

An issue of importance in clinical dentistry is the assessment of a tooth's viability. Because of the inherent difficulties in current methods of assessment, optical techniques are being explored as alternatives. Tooth viability is being examined by developing a system which can detect the presence of hemoglobin in the tooth by its absorption characteristics at 585 nm. This system uses a high intensity halogen light source and two narrow band interference filters to measure the ratio of the light intensity exiting the tooth at 585 nm to that at 595 nm. Light is delivered to the tooth by a fiber optic bundle and collected by a bifurcated bundle. An analogue divider continuously computes and displays the ratio of light intensity at the two wavelengths.

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OBJECTIVES: The purpose of this investigation is to explore the use of optical measurement techniques for routine clinical assessments in dentistry. Specifically, the detection of hemoglobin within the tooth by its realitive absorption at a wavelength of 585 nm.

METHODS EMPLOYED: A series of measurements has been performed on freshly extracted human molars to determine the relative change in absorption which occurs during the breakdown of hemoglobin. Based on these results an instrument suitable for clinical use has been developed. The completed device consists of a high intensity halogen light source which illuminates the tooth via a fiber optic cable. Light exiting the tooth is collected by a bifurcating fiber optic bundle which delivers the light to a set of narrow band interference filters. The filters have 10 nm passbands centered at 585 nm and 595 nm. The ratio of the light intensities at these two wavelenghts is a measure of the amount of hemoglobin within the tooth. By using two closely spaced wavelengths problems arising from significantly different optical paths which result from wavelength dependent refraction and scattering can be avoided.

MAJOR FINDINGS: The test on extracted molars show the expected decease in the relative amount of light absorbed at 585 nm as the hemoglobin breaks down. Based on the information obtained in this study, a system suitable for clinical trial has been developed.

SIGNIFICANCE: In present clinical practice the evaluation of a tooth's viability is made by applying an electrical current to the tooth. Perception of this stimulation by the patient is used as a positive indication that the tooth is viable. It is not always clear, however, that the perception is caused by the stimulation of the nerve within the tooth. Stimulation of nerve fibers in adjacent teeth or the surrounding tissue can lead to a false positive. Additionally loss of neural irritability may occur without loss of circulation and thus produce a false negative. Successful development of an optical system which directly measures hemoglobin content will provide an independent measure of tooth viability which is specifically dependent on the parameter of interest i.e. internal perfusion of the tooth.

PROPOSED COURSE: The clinical system will be used to evaluate a representative population of teeth to determine a population profile upon which clinical assessment of tooth viality can be based.

PUBLICATIONS

Webber, R., Maxwell, G.M.: "Colorimetric determination of tooth vitality," presented at The Annual Session of the American Association for Dental Research 1986.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RS 10203-02 BEI

80

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Clinical Assessment of Arterial Mechanical Properties

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

G.M. Maxwell, Senior Staff Fellow, BEIB, DRS
R. Chadwick, Biomedical Engineer, BEIB, DRS
D. Goldstein, Senior Investigator, IR, HE, NHLBI

COOPERATING UNITS (if any)
IR HE, NHLBI

LAB/BRANCH
Biomedical Engineering and Instrumentation

SECTION
Mechanical Engineering Section

INSTITUTE AND LOCATION
DRS, National Institutes of Health, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.3	.3	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this research is to develop an appropriate instrumentation system and associated data analysis techniques for evaluating the in-vivo mechanical properties of the brachial artery. Of particular interest is arterial distensibility, which can be evaluated in several ways. The most straightforward technique involves simultaneous measurement of arterial pressure and vessel diameter. An indirect and qualitative approach is also possible by analysis of the velocity waveform, a technique which can be performed non-invasively. Additionally, there are several theoretical formulations for arterial distensibility. Work on this project will be directed towards evaluating these various techniques to determine the limitations and advantages of each and to develop an instrumentation system suitable for routine clinical assessment of arterial mechanical properties.

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OBJECTIVES: It is the goal of this project to develop the necessary instrumentation and data analysis techniques to evaluate clinically the mechanical properties of the brachial and/or radial artery.

METHODS: The measurement of distensibility has been made in three ways. The first method computes distensibility from measurements made with an internal pressure transducer and diameter measurement made with an ultrasonic echo tracking system. Because it would be desirable from a clinical standpoint to have a non-invasive technique, the second method employs an indirect determination of the distensibility. This method measures the wave speed by assessing the relative arrival time of a doppler flow pulse at two different points along the vessel.

MAJOR FINDINGS: The measurement of arterial vessel diameter by the ultrasonic tracking is difficult. Extreme care must be taken to avoid artefacts cause by relative motion of the transducer and the subject's arm. Additional care is necessary to correctly identify the echos arising from the vessel walls since there are numerous echo producing interfaces in the intervening tissue. The situation is further complicated by the presence of double echos produced by a second reflection of the ultrasonic echo pulse off the transducer face. However, these secondary echos are useful since they will achieve their maximum amplitude when the transducer is positioned at right angles to the vessel wall and can thus be used to guarantee a good alignment of the transducer and vessel.

Measurement of the time delay between the arrival of the doppler velocity pulse at two different points is very sensitive to repeatable selection of the foot of the waveform. However, preliminary clinical measurements have indicated that it may be possible to do this with a reasonable degree of reliability.

SIGNIFICANCE: Results from the direct measurement of distensibility will permit the verification of theoretical predictions of the relationship between arterial mechanics and hypertension. Development of a non-invasive velocity system will permit the routine clinical evaluation of these properties and the effects of various therapies on the treatment of hypertension.

PROPOSED COURSE: Future efforts will be directed at constructing an appropriate fixation system for the arm and attendant fixturing for the transducers in order to make clinical measurements of arterial diameter. Bench experiments will be performed to evaluate the reliability of the velocity wave technique as a non-invasive alternative to evaluate arterial distensibility.

PUBLICATIONS

Maxwell, G.M.: Preparatory Measurements for Arterial Distensibility", Proceedings of the INSERM/NIH workshop on cardiovascular mechanics

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RS 10204-02 BEI

82

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Cell Handling Studies

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)
S.B. Leighton, Mechanical Engineer, BEIB, DRS
J.A. Berzofsky M.D., DCBD, NCI

COOPERATING UNITS (if any)
DCBD, NCI

LAB/BRANCH
Biomedical Engineering and Instrumentation

SECTION
Mechanical Engineering Section

INSTITUTE AND LOCATION
DRS, National Institutes of Health, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.3	.3	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

It is desireable to handle and process individual cells for monoclonal antibody work and other research endeavors. We are investigating a number of modalities for this, including flow cytometry, micromanipulators, dielectrophoresis, micropipettes, microtitre trays, and tissue culture techniques. We are considering sorting, storing, transporting and selectively inactivating cells. In addition, we are considering magnetic, gradient density, antigen-antibody, and solubility difference methods as possible candidates for separation techniques.

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OBJECTIVES: To provide a means of identifying and handling cells chosen by suitable criteria for successive stages of monoclonal antibody work.

METHODS EMPLOYED: Preliminary tests have been performed using a number of design concepts. A design is being developed.

MAJOR FINDINGS: There are as yet no major findings, as this work is still in the preliminary stages.

SIGNIFICANCE: This work, if successful, could lead to improving the yield of monoclonal antibody derivations.

PROPOSED COURSE: Less promising techniques will be eliminated from consideration, and the most promising ones will be further refined into preliminary designs for evaluation.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 RS 10205-02 BEI

PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Constant Shear Field Apparatus for ESR Studies

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

S.B. Leighton, Mechanical Engineer, BEIB, DRS
H. Kon, Research Chemist, LCP, NIADDK

COOPERATING UNITS *(if any)*

LCP, NIADDK

LAB/BRANCH

Biomedical Engineering and Instrumentation

SECTION

Mechanical Engineering Section

INSTITUTE AND LOCATION

DRS, National Institutes of Health, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.2	.1	.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Electron Spin Resonance (ESR) is a valuable technique for studying membrane bound proteins. In particular, it can be used to study labeled proteins in red blood cell membranes. In order to know the orientations of the proteins with respect to the membranes, it is necessary to orient the membranes with respect to the ESR device. A fluid shear field serves the purpose of orientation, since the red blood cells are discoid and distensible. However, flow in capillary tubes, although functional and simple, provides a non-uniform shear field due to the Poiseuille velocity distribution. We are planning to use a moving thin polyester tape to create a uniform shear field and hence sharper resolution of orientation and a better signal to noise ratio.

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OBJECTIVES: To provide a uniform shear field in a small sample of erythrocyte or other suspension, compatible with the D.C. magnetic and R.F. magnetic and electrical fields of the ESR apparatus, and to have a precisely controlled shear field.

METHODS EMPLOYED: A plastic housing has been constructed to fit within the 11 mm. diameter x 25 mm. long microwave cavity of the ESR instrument. This housing holds the sample suspension and provides precise guides for a mylar tape that passes through the housing, turns 180 degrees and travels simultaneously past itself in the opposite direction. Three regions are formed, bounded by the walls of the housing and the two sections of tape. Shear rate is, determined by the spacings in the device and the speed of the tape.

MAJOR FINDINGS: The apparatus is complete, and the fluid flow characteristics have been tested and found to be as required expected. There is a uniform flow, with constant spacing between the tapes. Instabilities have not been observed.

SIGNIFICANCE: This device, should allow ESR investigators better resolution in their measurements.

PROPOSED COURSE: The microwave section of the instrument must be built and tested. The shear field will be examined by observing the alignment of the erythrocytes microscopically, through observation ports planned for that purpose. A computer interface for control of the experiment is planned.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RS 10206-02 BEI

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Concentration Uniformity by Diastolic Phasing of Pulsed Intra-Arterial Infusions

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)
D.R. Shook, Biomedical Engineer, BEIB, DRS
J.L. Doppman, Chief, DRD, CC

COOPERATING UNITS (if any)
Diagnostic Radiology, CC (J.L. Doppman); Chemical Engineering Section, BEIB, DRS (R.J. Lutz)

LAB/BRANCH
Biomedical Engineering and Instrumentation

SECTION
Mechanical Engineering Section

INSTITUTE AND LOCATION
DRS, National Institutes of Health, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.00	0.55	0.45

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Inadequate infusate/blood mixing during intra-arterial chemotherapy at low catheter infusion rates (1 ml/min) produces highly nonuniform downstream drug concentrations. Inadequate treatment of the lesion and overexposure of normal tissues may result. Studies are underway to investigate the advantage of diastolically phased, high flow rate, pulsed intra-arterial infusions in effecting uniform downstream concentrations. Triggered by the ECG, a high velocity pulse during the interval of lowest blood velocity and retriggered on successive cardiac cycles, will yield average flow rates at the desired therapeutic value. Apparatus to produce that pulsed flow and methods of *in vivo* evaluation of downstream concentration uniformity are being developed. Highly realistic flow studies in an accurate model of the superior carotid artery and cerebral branches have demonstrated high essentially complete concentration uniformity as determined by spectrophotometric comparison of infusate dye concentrations in all downstream branches. The apparatus has been optimized for animal and clinical use, and pulsed intra-arterial infusions have been used in low flow rate clinical chemotherapeutic treatments, and in amimals for clinical evaluation.

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OBJECTIVES: Evaluate effectiveness of diastolically phased, pulse intra-arterial infusions in attaining uniform downstream drug concentrations in chemotherapy.

METHODS: Conventional end-hole catheters were placed in an acrylic model of the human cerebral circulation. Effluents from five brances are collected and infusate dye concentration individually determined spectrophotometrically. The degree of variation from the mean concentration indicates the severity of streamline channeling into particular branches. Infusate is pulsed once per cardiac cycle, and phasing controlled by an adjustable delay. Pulse control parametrers, i.e., average infusion rate, pulse infusion rate, pulse width, beat/pulse ratio, and delay, are altered using a modified angiographic injector (Medrad, Inc., Pittsburgh, PA.) coupled to a fast response solenoid valve.

MAJOR FINDINGS: In vitro studies indicate very good mixing and concentration uniformity due to pulsed intra-arterial infusions phased during the interval of lowest blood velocity. Studies were conducted in a realistic vascular model of the superior internal carotid artery and major branches by spectrophometrically evaluating dye concentration in branch arteries.
Both the pulse infusion rate and phasing are effective in producing highly uniform concentrations. Pulsing can decrease the nonuniformity due to streaming of steady infusions by 90%. Diastolically phased pulsing offers a 70% decrease over nonphased pulsing and high pulse infusion rates can decrease streaming effects by 75% over low pulse infusion rates. Rapid acceleration to higher rates, however, can cause catheter tip recoil. Tip placement must therefore be verified radiologically under the infusion conditions. Catheter material compliance and the catheter tip placement, both longitudinally and across the vessel lumen, can significantly alter uniformily measurements if appropriate pulse parameter settings are not chosen.

SIGNIFICANCE: The possibility of streaming during intra-arterial chemotherapeutic infusions has been recognized. Low catheter infusion rates (1 ml/min) do not disturb the laminar nature of the blood flow and therefore allow the infusate to follow a restricted set of laminae. As downstream branches in the vasculature divide the flow, this restricted set of laminae may remain undivided and flow into a side branch, sparing the intended vessel and downstream lesion of effective therapy. At best, the downstream drug concentrations are markedly nonuniform. By exploiting the inherent mixing of high flow rate infusions (high relative velocities) with the circumfluent blood flow, complete uniformity can be achieved. Average flow rates in the therapeutic range (desired administration schedules) are maintained by catheter delivery of these short, high velocity pulses of infusate during the interval of lowest blood flow within successive cardiac cycles, and triggered by the ECG.

PROPOSED COURSE: The apparatus will be applied in animal and clinical studies. The animal studies will show the degree of in vivo efficacy.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RS 10207-02 BEI

B8

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Clinical Engineering Program for the Clinical Center Patient Areas

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
R. Corsey, Electronic Engineer, ACES, BEIB
L. Eldridge, Associate Hospital Administrator, CC
C. Strong, Environmental Safety Officer, CC
J. Harrison, Building Services Manager, CC
R. Bruun, Associate Hospital Administrator, CC
J. Decker, Director, CC
S. Galen, Associate Hospital Administrator, CC

COOPERATING UNITS *(if any)*
Clinical Care Instrumentation Section, DRS, NIH
Nursing Department, CC, NIH

LAB/BRANCH
Biomedical Engineering and Instrumentation

SECTION
Applied Clinical Engineering

INSTITUTE AND LOCATION
DRS, National Institutes of Health, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.4	0.15	0.25

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A comprehensive program of clinical engineering is provided for all patient-care areas of the Clinical Center. This consists of selecting equipment testing standards, designing test equipment, performing safety and performance tests, assuring hospital compliance with applicable standards of the Joint Commission on Accreditation of Hospitals, assuring hospital compliance with other pertinent standards such as the National Electric Code and the Standard for Health Care Facilities,investigating electric shocks to patients and employees, providing engineering expertise to hospital standing committees such as the Clinical Center Safety Committee and the Clinical Center Standardization Committee (equipment), providing education in the form of lectures seminars and "in service" demonstrations, designing and modifying equipment for clinical and research use, correcting equipment malfunctions through interface with manufacturers and the Center for Devices and Radiological Health, FDA, and the Emergency Care Research Institute and participation in patient care product recalls and seizures.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RS 10208-02 BEI

PERIOD COVERED: October 1, 1985 to September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.): Hemodynamic Principles in the Control of Coronary Blood Flow

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation):

Thomas L. Talbot, MS, ACES, BEIB
Stephen E. Epstein, M.D. Cardiology, NHLBI
Richard O. Cannon, III, M.D. Cardiology, NHLBI

COOPERATING UNITS (if any): Cardiology Branch, NHLBI

LAB/BRANCH: Biomedical Engineering and Instrumentation

SECTION: Applied Clinical Engineering

INSTITUTE AND LOCATION: DRS, National Institutes of Health, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.75	.75	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The effects of atherosclerotic epicardial stenosis on coronary vascular resistance can be understood in terms of basic fluid mechanical principles. Resistance is directly related to the trans-stenotic pressure drop and inversely related to flow. However, even with a fixed anatomic stenosis, resistance is not fixed; it increases as trans-stenotic flow increases. This exacerbates the pressure drop across the stenosis that develops as a result of flow; at high flows, large pressure drops can occur. This characteristic of flow through stenotic lesions can contribute to a "steal" physiology, between either epicardial or intramural coronary arteries. Studies have also shown the clinical importance and influence of dynamic alterations in coronary resistance, occurring either at the large or small vessel level. In addition, compressive forces exerted by the myocardium or by elevated intraventricular pressures can increase coronary vascular resistance, and therefore can interfere with myocardial perfusion. All these factors must be considered in order to understand the mechanisms leading to myocardial ischemia, and therefore to the clinical syndrome of angina pectoris.

Further studies have shown the complex interaction of the coronary vessels and the peripherical vascular bed. The vessels of each group were treated as resistance vessels either in series or in parallel configuration. The results have demonstrated dramatically the individual and collective contribution of these vessels towards the maintenance if sufficient myocardial perfusion.

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ZO1 RS 10208-02 BEI

OBJECTIVE: Increase the clinician's understanding of the physics involved in the restriction of blood flow in order to promote a more in-depth understanding of the physiology of certain coronary disorders.

METHODS EMPLOYED: Mathematical treatment of simple restrictions in vessels as simple luminal narrowings in rigid tubes. Present results of calculation graphically and simply at first and subsequently increasing the complexity of the governing equations to encompass second and third order effects. Experimental data from dogs will serve as tests of hypotheses reached from the mathematical calculations.

SIGNIFICANCE Often myocardial ischemia occurs when the classical evidence of vasoconstriction would not indicate a severe decrease in coronary blood flow. A more thorough understanding of the factors that effect coronary blood flow will provide the clinician with a better ability to detect and treat potential ischemic conditions.

PROPOSED COURSE: To begin with simple first order calculation and measurements in models and eventually expand theory and experiments to predict and measure more detailed flow characteristic in the coronary arteries.

PUBLICATION: Epstein, S.E., Talbot, T.L. : Dynamic Coronary Tone in Precipitation, Execerbation and Relief of Angina Pectoris. The American Journal of Cardiology Vol. 48: 797-803 Oct 1981

Epstein, S.E., Cannon, R.O., Talbot, T.L. : Hemodynamic Priciples in the Control of Coronary Blook Flow. The American Journal of Cardiology 56:4E 1985

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RS 10209-02 BEI

91

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Evaluation of Disposable Blood Pressure Transducers

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
E.C. Walker, Mechanical Engineer, ACES, BEIB
T.L. Talbot, Mechanical Engineer, ACES, BEIB
R.E. Clark M.D. Chief, Heart Surgery, NHLBI
D.P. Dolan, CC, CCM

COOPERATING UNITS *(if any)*
NHLBI, Heart Surgery
CC, ICU
CC, Critical Care Medicine

LAB/BRANCH
Biomedical Engineering and Instrumentation

SECTION
Applied Clinical Engineering

INSTITUTE AND LOCATION
DRS, National Institutes of Health, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.25	.25	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A sample of each manufacturer of disposable blood pressure transducers was tested for compliance to AAMI's specifications for these devices. The data are currently being compiled for presentation to those responsible for placing these devices in use throughout the Clinical Center. The data do not represent an official endorsement of a particular device.

PHS 6040 (Rev. 1/84) GPO 814-018

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OBJECTIVE: To define the best disposable transducer(s) for use in the various intramural applications.

METHODS: The transducers are tested in a temperature and humidity controlled chamber. All specifications for such devices are tested using standard industrial testing techniques. All data are taken with the aid of a small computer for storage and subsequent analysis.

SIGNIFICANCE: Due to the burgeoning types of disposable pressure transducers, careful consideration of specification and reliability of quoted specification must be made before safely introducing a device into the varied areas throughout the Clinical Center.

PROPOSED COURSE: Tests have been completed on transducers from major manufacturers. Preliminary tests have been performed on devices prior to introduction into patient areas. Additional tests on devices with high priority will be performed to identify potential performance problems.

PUBLICATIONS:

Walker, E.C., Talbot, T.L., Seipp, H.W., Sweeney, W.V., Dolan, D.P., and Clark, R.F.: A preliminary assesssment of disposable blood pressure transducers. Advances in Bioengineering, Dec. 1984.

Walker, E.C., Lawson, D., Talbot, T.L., and Dolan, D.P.: An engineering evaluation of disposable resistive bridge type blood pressure transducers, Proceedings AAMI 20th Annual Meeting, May 1985.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RS 10210-02 BEI

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Electronic Patient Monitoring System

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)
H.E. Cascio, Electronic Engineer, BEIB, DRS
G. Hemphill, Engineering Technician, BEIB, DRS
K. Musallam, Head Nurse, CCMURS
D. Cirelli, Administrator Officer, CC, OD

COOPERATING UNITS (If any)
CCOD

LAB/BRANCH
Biomedical Engineering and Instrumentation

SECTION
Electrical and Electronic Engineering Section

INSTITUTE AND LOCATION
DRS, National Institutes of Health, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.8	0.3	0.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

An electronic patient monitoring system that alerts the nursing staff when a patient (or patients) is leaving the nursing unit is a welcome aid in the care of Alzheimer's Disease patients and other patients with dementing illnesses. The system design is based on a wrist watch size radio transmitter, worn by the patient. A detector unit, placed at the two doors, senses the presence of any patient wearing a transmitter watch. With the detection of a patient's transmitter, a microprocessor based controller locks the exiting door, activates an alarm, and displays the patient's name and location on a computer monitor screen. The personal computer would record the time and date for each patient's attempt to leave. This data would then be used for statistical analysis.

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OBJECTIVES: To design and fabricate a system that alerts the nursing staff and locks the exit door when any patient wearing a transmitter watch attempts to leave the nursing area. Also interface the Electronic Monitoring System to a personal computer, so that the computer will function as a system input and output devcice and record patient behavioral patterns.

METHODS EMPLOYED: A miniaturized battery powered radio transmitter was constructed and mounted in a plactic watch case. The transmitter, worn by each patient, has a broadcast range of six feet. Each transmitter is modulated with a different frequency so that each patient can be identified. The two doors leading out to the nursing unit are monitored by placing a modified broadcast radio above each door. The carrier frequency of each radio transmitter is 1720 KHz (just above the broadcast band). The detected signal from the patients' transmitters are fed to a comb filter, which identifies which transmitter is being sensed. A microprocessor based controller, then alerts the nurses by activating an alarm and displaying the name of the wandering patients and their location on a computer monitor screen. The controller would also activate a door locking mechanism when a patient enters the sensing area. The locking of the doors on the nursing unit is permissible since the power for operating the locks is obtained from the Clinical Center fire alarm power system.

SIGNIFICANCE: The task of keeping the demented disease patients safe in a strange environment, and from becoming lost within the Clinical Center would greatly aid the nurses.

PROPOSED COURSE: A four patient monitoring system has been installed and is now in operation. The system is now under going evaluation and reliability testing. Presently, work is under way to install the door locks, interface a PC computer to the system, and increase the number of monitoring channels to eight.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RS 10211-02 BEI

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
IEEE-488 GPIB and Personal Computer Instrumentation Use Program Development

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

T.R. Clem, Sr., Electronic Engineer, EEES, BEIB, DRS

COOPERATING UNITS (If any)

LAB/BRANCH
Biomedical Engineering and Instrumentation Branch

SECTION
Electrical and Electronic Engineering Section

INSTITUTE AND LOCATION
DRS, National Institutes of Health, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.25	0.2	0.05

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The increased availability and reduced cost of the small Personal Computer has created increased interest in automating data acquisition and process or experiment control in areas where such things were not feasible before due to cost or complexity. With these changes also came a significant increase in the use of the IEEE-488 GPIB by instrument makers. By combining the two, sophisticated instrumentation and data acquisition systems can be assembled quickly and inexpensively. The BEIB is continuing to develop the expertise to provide guidance and assistance to requirements where this approach provides the optimum solution. This capability is further assisted by the BEIB-SERP specifying the IEEE-488 interface on new equipment acquisitions whenever possible. The increase at the NIH in the numbers and use of the IBM PC has made this capability of great value to the NIH Intramural Research program.

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OBJECTIVE: Continue to develop expertise in the Branch in the use of IEEE-488 GPIB equipment, instruments, and controllers and recommend bus-compatible instruments for acquisitions by the SERP in order to be prepared to respond quickly to requests for an instrumentation systems.

METHODS EMPLOYED: Generally an instrumentation system will be assembled around the IEEE-488 GPIB for one of four reasons: 1) a prototype system is desired to prove feasibility before a dedicated system is designed and built, 2) a temporary system is needed to satisfy an urgent, short term need, such as replacing a dedicated system that is out of operation for maintenance or repair purposes, 3) to expedite data acquisition into a computer system for further analysis, or 4) to produce a system which is inherently flexible enough to adapt to a wide range of potential future needs.

SIGNIFICANCE: The capability of assembling an instrumentation system with a controller and GPIB-controlled instruments allows the EEES to provide a rapid response to an investigator's call for instrumentation. By assembling a system with "off-the-shelf" instruments from the SERP, the cost of special-purpose measurement or control systems can be kept quite low. If the experiment is a short-term project the instruments can be returned to the SERP with virtually no expenditures, by the investigator, for equipment.

PROPOSED COURSE: Continue to maintain state-of-the-art capability in the field of bus-compatible instruments and controllers. Seek to make this capability better known and understood around NIH. Expand capability which includes IBM PC's as controllers, and integrating systems with other types of PC-based I/O hardware. Develop expertise in assembling instrumentation systems using PC's and commercially - available laboratory acquisition and analysis programs.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RS 10212-02 BEI

PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Fluoroimmunoassay Apparatus

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

W.S. Friauf	Chief, EEES	BEIB, DRS
R.L. Berger	Physicist	LTD, NHLBI
G. Hemphill	Electronic Tech.	BEIB, DRS

COOPERATING UNITS (If any)

CDC, Atlanta

LAB/BRANCH

Biomedical Engineering and Instrumentation Branch

SECTION

Electrical and Electronic Engineering Section

INSTITUTE AND LOCATION

DRS, National Institutes of Health, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.3	0.2	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

New rare earth chelate fluorescent probes with a decay time constant much longer than the background decay time-constant of typical organic materials offer the potential of a great improvement in sensitivity of time resolved fluorimetry. However, to rival radioimmunoassay methods the required sensitivity improvement is so great that overload recovery of the fluorescence detector is a major problem. Extensive past work on this problem is largely inapplicable to the very low speed and level requirements of this situation. Consequently initial effort is being applied to determining the optimum fluorescence detection device and ancillary signal overload limiting circuitry. Problems related to the fluorescent probe at extremely low sample concentrations will be studied in collaboration with CDC, which is interested in this approach as another tool for AIDS research. Finally an evaluation of the details of excitation, optical filtering, and digital signal processing will be worked out and optimized.

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OBJECTIVES: To develop a convenient, economical bench top instrument capable of performing fluoroimmunoassays with sensitivity comparable to or possibly even better than radioimmunoassay methods in current use.

METHODS EMPLOYED: The speciman is tagged with a rare earth chelate fluorescent probe and illuminated with light pulses at 337nm from a compact nitrogen laser with a sealed plasma unit. The fluorescense is sensed with a red sensitive photo multiplier tube with photon counting electronics. Background fluorescence is gated out and then the slower decaying probe fluorescence is integrated by a digital counter.

SIGNIFICANCE: If the theoretical potential of this approach can be realized, fluoroimmunoassays could replace radioimmunoassays for much biological testing, with a great improvement in convenience and safety.

PROPOSED COURSE: Continue to improve sensitivity, with particular emphasis on reducing detector overload and hysterysis by optimizing optical filtering and by electronic means. Also background noise must be reduced further.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RS 10213-02 BEI

PERIOD COVERED: October 1, 1985 to September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.): Vitreous Fluorophotometry Analysis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.M. Bungay	Chemical Engineer	BEIB, DRS
R.F. Bonner	Biophysicist	BEIB, DRS
M.S. Roy	Ophthalmologist	CB, NEI
M.J. Podgor	Statistician	BE, NEI

COOPERATING UNITS (if any): Clinical Branch, NEI; Office of Biometry & Epidemiology, NEI

LAB/BRANCH: Biomedical Engineering and Instrumentation

SECTION: Chemical Engineering Section

INSTITUTE AND LOCATION: DRS, National Institutes of Health, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	0.5	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Vitreous fluorophotometry (VFP) is a clinical procedure for measuring the spatial distribution of fluorescence along the optical axis in a human subject's vitreous following administration of a fluorescent dye. In this project the clinical instrument is a Fluorotron Master manufactured by Coherent, Palo Alto, CA, and the dye is sodium fluorescein given by intravenous injection. The purpose of the project is to improve the protocols for conducting the measurements, processing the output from the instrument and analyzing the results from the population of normal volunteers and patients.

One disease to be considered is diabetes and the associated pathology diabetic retinopathy. The goals are to evaluate the procedure for use in (1) screening for early detection, and (2) monitoring for treatment efficacy. A pharmacokinetic mathematical model has been developed which describes the rate of appearance of fluorescein in the vitreous in terms of the blood-retinal barrier permeability (characterized by permeability coefficients of the retina-vitreous interface) and transport of fluorescein within the vitreous by diffusion (characterized by a diffusion coefficient). Plasma protein binding of fluorescein is also incorporated. Measurements have been made on 22 normal volunteers to evaluate the performance of the instrument, establish baseline parameter ranges, and critique existing protocols/analysis schemes.

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OBJECTIVES: The objective is to evaluate vitreous fluorophotometry through improving the protocols for conducting measurements, processing the instrument output and analyzing the results from patient populations and normal controls. The intent is to extend the evaluation in the direction of discerning the procedure's usefulness in (1) screening for early detection of retinopathy and/or (2) monitoring the effectiveness of treatment in slowing the progression or reversing the development of blood-retinal barrier alterations.

METHODS EMPLOYED: A Fluorotron Master, manufactured by Coherent, Palo Alto, CA, is being used in the evaluation. This instrument was designed for clinical use. However, judging from the variety of protocols that have been proposed, the developers had not optimized the procedures and there is considerable skepticism in the research community about the instrument. Consequently it was deemed necessary to perform existing procedures on a population of normal volunteers coupled with the development of mathematical model to understand the normal in vivo phenomena involved, as well as the response characteristics of the instrument.

The existing procedures involve the single bolus injection of sodium fluorescein into an antecubital vein at a dose of 14 mg/kg. Blood samples are withdrawn from the contralateral vein at four time points over the succeeding 90 minutes in order to measure the decay of plasma levels of fluorescein and the variation over time in the percent of fluorescein binding to plasma proteins. The instrument is used to measure the spatial distribution of fluorescence within each eye at four time points: before fluorescein injection and nominally at 3, 30 and 60 minutes post-injection. The instrument illuminates the interior of the eye with a narrow, off-axis beam of monochromatic light and detects emitted light rays which follow a similar narrow path oriented at a 14° angle from the illuminating beam. The intersection of the two beams forms a narrow diamond shaped volume whose major diagonal is under 2 mm in length and is oriented along the optical axis. By means of automated adjustable optics the position of the sampling volume is moved over a distance of 3.5 cm along the optical axis in increments of 0.25 mm. Each such scan takes about 12 seconds. By means of comparison to an internally mounted fluorescence standard which has been calibrated with respect to solutions of known fluorescein concentration, the instrument computes the concentration of fluorescein equivalent to the fluorescence detected in the sampling volume at each scan step. The instrument is also used to determine the fluorescence in the plasma samples by means of an adaptor which holds a curvette in the scan path.

The mathematical model represents the vitreous as a homogeneous spherical medium enclosed by a thin membrane which is bathed by plasma. The thin membrane models the retinal vessel walls and the pigmented epithelium which are collectively referred to as the blood-retinal barrier (BRB), albeit with an area corresponding to the membrane surface, not the anatomical surfaces. The plasma-vitreous exchange of fluorescein is taken to be a passive process driven by the difference in concentration of free fluorescein adjacent to the two sides of the membrane. However, a flux of fluorescein from vitreous to blood proportional to the interfacial vitreous concentration is superimposed on the passive exchange because of reports in the literature of a unidirection retinal fluorescein pump. Transport within the vitreous is currently assumed to be by pure diffusion.

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MAJOR FINDINGS Analysis of measurements on a population of 22 normal volunteers, including six who were measured again on a different day, led to several findings. All of the existing analysis protocols have deficiencies. A major difficulty which had not been optimally addressed was the spatial averaging of the fluorescence due to the size of the fluorotron sampling volume. This averaging gives rise to output fluorescence readings which are distorted representations of the local fluorescence. We have developed an analysis scheme which uses a microcomputer to simulate the instrument output by an analogous spatial averaging of the vitreal fluorescein concentration profile generated numerically from the mathematical modes. A regression routine determines the values of the blood-retinal barrier permeability and vitreous diffusivity which minimize the difference between the experimental and simulated fluorescence scans.

SIGNIFICANCE: The shape of the fluorescence profiles and the rate of progressive changes in the profile are indicative of the underlying transport mechanisms are expected to occur in pathological conditions. For instance, diabetes, or at least diabetic retinopathy, has been reported to lead to greatly enhanced leakiness of the BRB to fluorescein. Diabetic retinopathy are experimental. It could be beneficial to have a relatively simple and benign procedure for determining leakiness of the BRB which might be correlated with the progression of the disesase, and which is likely to be more sensitive than existing diagnostic procedures, such as visual inspection of the retina. New diagnostic procedures could be used to follow the effectiveness of treatment regimes in slowing the progression of disease for reversing its developmnent. Alternatively, the diagnostic procedure could be used as an early screening tool to determine when to commence treatment.

PROPOSED COURSE: We will apply our analysis scheme first to the scans obtained from the normal volunteers to standardize the analysis protocol and determine the normal ranges for the parameters. We will then apply the scheme to the scans from the diabetic patient population. The diabetics have been subdivided according to other measures into groups with varying stages of development of retinopathy ranging from no retinopathy to clearly established retinopathy. The objectives will be to determine whether changes is permeability do correlate with the progression of retinopathy and, if so, at what stage can abnormal pfermeability values be distinquished from the normal range.

PUBLICATIONS: Roy,M.S, Bonner, R.F., Bungay, P.M., and Podgor, M. : Posterior Vitreous Fluorophotometry with use of the Fluorotron Master: Normal subjects. Archives of Ophthalmology. (In Press)

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RS 10214-02 BE.I

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Photoradiation Therapy

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	P.D. Smith	Physicist	BEIB	DRS
Others:	R.F. Bonner	Biophysicist	BEIB	DRS
	A. Russo	Section Chief	ROB	NCI
	M. Manyak	Staff Fellow	ROB	NCI
	D. Mathews	Veterinarian	VRB	DRS
	H. Pass	Surgeon	SURG	NCI

COOPERATING UNITS (if any)
ROB, NCI; VRB, DRS; SURG,NCI

LAB/BRANCH
Biomedical Engineering and Instrumentation Branch

SECTION
Electrical and Electronic Engineering Section

INSTITUTE AND LOCATION
DRS, National Institutes of Health, Bethesda, Md 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	2.5	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Photodynamic therapy (PDT), which is the interaction of light with hematonorphrin derivative (HDP, Photofrin II), is being studied for possible clinical use in several areas. Currently, PDT is being investigated in the following: cancer of the prostate (beagles); carcinoma in situ of the bladder and urinary tract (foxhounds); and pulmonary metastatic disease (nude mice). Specially designed fiber optic probes and and introducers were manufactured for all cases, and calibrated in terms of irradiance delivered to the tissue. Photosensitised skin is a side effect of PDT, and the possibility of using agents to protect against this photosensitivity was addressed. In particular, hydrochlorothorazide appears to have a pronounced effect on reducing skin photosensitivity in nude mice models. Dosimetry for PDT is complex due to the variable irradiation levels within the tissue, sequestering of HDP, and tissue oxygenation. To address the first of these points and to permit light distribution within tissue to be formulated, measurements were made of light emanating from tissue as a function of radial distance from the point of injection of the light into the tissue. Theoretical analyses permit extraction of two parameters, the mean free path length between isotropic scattering events and the absorption coefficient, with and without the presence of HDP, for each of the tissues studied. These include muscle, bladder wall, liver, kidney, skin, etc. For these tissues, flux levels as a function of distance from the point of light injection have been established for three different wavelengths, 514nm, 660nm, and 780nm.

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OBJECTIVES: To evaluate the prospect of PDT as a modality for treatment of selected diseases. To enhance the effectiveness of PDT as a clinical procedure by reducing the complications associated with its usage. To characterize tissue permitting theoretical analyses to be performed which predict the irradiation level at any point in the tissue distant from the point of light injection.

METHODS EMPLOYED An argon ion laser pumped a liquid dye laser (Coherent model PRT-100) producing up to 5W at 631nm. Fiber optic probes were developed from QPC PCS200 and QPC PCS600 fused silica fibers. For the bladder and prostate work, the PCS600u fiber was stripped of the jacket and cladding for the final 2cm of the fiber. The bare end was then coated with titanium dioxide impregnated latex which produced a uniform scattering distribution along the length of the 2cm tip. In the prostate, an introductory translucent sheathing was used for fiber insertion which precluded blood from coming into contact with the fiber. Fibers were inserted either trans-perineally or directly into the exposed prostate, and in the former, fiber positioning within the prostate was augmented with the aid of trans-rectal ultrasound scanning. Input power at 631nm was maintained below 200mW for 30 minutes. In the ureter, the fiber was inserted by exposure of the orifice to the bladder, whereas external entry was gained to the urethra. Exposure levels were obtained of 100mW at 631nm for 7 minutes at the 2cm long tip of the fiber. For the urethra treatment, a 2.5 mm angioscope was used to examine and record on video tape the irradiated region. For the murine study, QPC PCS200u fibers were perpendicularly cleaved and the tip was inserted into a 27 gauge chest tube. The lung had previously been collapsed and an intralipid scattering solution introduced to promote uniform irradiation of the pleural surface.

Skin photosensitivity was studied by irradiating the skin surface of nude mice which were free to move around in a clear bottomed container. A specially constructed table allowed the mice to be irradiated from below using a projector which was modified to permit attenuation of the infra-red by a copper sulphate filter. Irradiating levels were assessed using an EG&G radiometer and typical exposures of $0.4mW/cm^2$ for 15 minutes were used.

A pair of optical fibers were used to inject light of a given wavelength (514nm, 660nm, or 780nm) into tissue at one point and to measure the light emanating from the tissue at known distances from the point of injection. Several tissues were examined, both with and without the presence of HPD; these include skin, muscle, bladder wall, kidney, and liver.

MAJOR FINDINGS: The canine prostate model tolerates the direct injection of 10mg/kg of Photofrin II followed by 30min of 631nm irradiation at $150mW/cm^2$ with no apparent complications. At fiber input levels of 200mW or greater, drying out of blood droplets adhering to the fiber within the introductory tube caused coagulation leading to localized heating due to deposition of light energy into the coagulated region. This is not a problem if the fiber is in contact with a continually wetted field; in addition, the introductory sheath, when modified to overcome the coagulation problem, also removed any concerns due to breakage of the fiber tip. There appears to be atrophy at 1 month of the treated lobe and histological sections indicate reduction and in some cases elimination of prostate cells.

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The murine model (i.v. injection of a DNA transfectant of a human lung adenocarcinoma derived cell line) was established to provide a method of studying pulmonary metastatic disease. This model manifests itself with multiple surface lesions. The mice in general did not tolerate the procedure well, and multiple deaths occurred as a result of the induced pneumothorax. Of the mice that were treated and survived, no evidence of the disease was found at autopsy. A nude rat model is being established along similar lines to overcome treatment problems asssociated with the small size of mice.

The major clinical complication associated with PDT is the sensitivity to light imparted to normal skin by the HPD. This can cause severe burns if appropriate attention is not paid to remaining in subdued lighting. Total exposure of $4J/cm^2$ was sufficient to cause burns on the skin surface of the mice preinjected (48 hours) with Photofrin II at 2.5mg/Kg. Preliminary evidence shows little or no protection provided by either -carotene or furosemide (Lasix), which were chosen for their singlet oxygen quenching and diuretic properties respectively. Hydrochlorathorazide, also a diuretic, was found to prevent any burning on the exposed skin surface.

The irradiation level falls off very rapidly as a function of depth into the tissue. At 631nm, PDT could be expected to treat to a depth of 5mm without producing surface irradiation levels leading to burns. For the argon line at 514nm, this depth is reduced to a few mm.

SIGNIFICANCE: Photoradiation therapy offers an alternative and in some cases, for example pulmonary metastatic disease, a unique approach to treatment of cancer. Future acceptance of PDT will depend on several factors, some of which are addressed herein. For example, to control the phototoxicity would promote the usefulness of the treatment, and to assess the exact level of irradiation is essential in designing zones of treatment. In addition, to identify clinically relevant diseases and define treatment modality for them is of prime import for PDT.

PROPOSED COURSE: To further investigate the animal models and where appropriate to initiate human clinical trials. Additional agents will be tested for reducing or eliminating the phototoxicity of HPD. Light penetrance studies will be incorporated with singlet oxygen detection methods to define the levels necessary to cause the cytotoxic effects of PDT.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RS 10215-02 BE1

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Measurement of Trace-Level Metals and Complexes in Biological Milieux

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)
A.F. LeRoy, Ph.D., BEIB, DRS
M. Linnoila, M.D., Ph.D., CPB, NIMH
K. Flora, Ph.D., PRB, NCI
M.K. Wolpert, Ph.D., Drug Eval. Br., DCT, NCI
P. Galle, Prof. of Biophysics, Medical Faculty, Creteil, France
B. Hecquet, Ph.D.. Centre Oscar Lambret, Lille France
P. Parsons, Ph.D., BEIB, DRS

COOPERATING UNITS (if any)
CPB-NIMH, DCT-NCI, PRB-NCI, Biophysics Dept., Med. Faculty, Creteil, France: Centre Anti-cancereux "Oscar Lambret", Lille, France

LAB/BRANCH
Biomedical Engineering and Instrumentation Branch

SECTION
Analytical Methods

INSTITUTE AND LOCATION
DRS, National Institutes of Health, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.20	0.70	0.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Analytical methods have been developed for quantitation and characterization of metallic elements and their complex species present in biological samples at trace levels.

Analysis of vanadium in samples from human subjects under study has been conducted. Results obtained have shown very low concentrations, in the range of nanograms-of-vanadium-per-gram of sample. The very small amounts of CSF samples available make reliable, reproducible results difficult to assure.

Platinum complex behavior is under study for several different platinum-containing complex compounds used clinically. Chromatographic techniques are used to make separations of platinum complex species (bound and unbound to protein) in human plasma at clinically used concentration levels. Material balances suggest that all the platinum in the several fractions can be accounted for quantitatively. Analytical methods developed have been used to make kinetic studies of behavior of platinum species from cisplatin (including the parent complex, cis-diamminedichloroplatinum II (CDDP), and its transformation products) in plasma and to relate them to quantitation of protein in each fraction collected. Atomic Absorption Spectroscopy with electrothermal atomization is used to quantitate total platinum or single isolated species.

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High Pressure Liquid Chromatographic (HPLC) separation with electrochemical detection using a modified polarographic analyzer permits quantitation of the parent CDDP complex in aqueous solutions with a detection limit of about 50 ppb.

OBJECTIVES: To develop and study analytical methods suitable for trace analysis of specific elements and their compounds and complexes in biological milieux. Emphasis has been on developing techniques for quantitation of elements that have important biological effects such as platinum, vanadium, nickel, cobalt, calcium and iron. More particularly, to conduct the study of the kinectics of transformations of metal-complex compounds used as anti-cancer agents and their transformation products in protein containing solution and in human body fluids, such as blood plasma, using improved techniques for identifying, characterizing and quantitating metal complex species in such samples. Some of these elements are found at trace level concentrations in normal biological tissues but they may also be found in abnormal concentrations in tissues in organisms in some disease states. In still other cases, they may be present as a result of therapeutic intervention.

METHODS EMPLOYED: Ion exchange chromatography and gel filtration have been used successfully as methods to resolve metal-ion complexes in in vitro studies. In vivo studies with patients being treated with cis-platin have involved quantitative analysis and characterization of the metal-complex species in urine using ion exchange chromatography.

The techniques have been applied to studies of the reaction in vitro to establish quantitative rate data describing the transformations. It was recognized from our early work that CDDP formulated as cisplatin with mannitol and NaCl (and HCl to adjust pH) is transformed in solution into charged and uncharged species. The present work was undertaken to make quantitative measures under differing conditions to measure the influence of [Cl] on the rate and extent of hydrolysis and to establish the feasibility of material balance studies for the various species and obtain the several species in sufficient quantity and purity to permit characterization. In addition, gel filtration techniques have been studied to establish the rates of binding of platinum species to proteins in body fluids.

HPLC techniques have been studied to establish their performance and characteristics. Detection using electrochemical methods has been adapted to supply a rapid, on-line technique with good sensitivity. In vitro studies with human tumors cultured in defined medium have been undertaken with Cisplatin, CBDCA, and TNO-6 to establish the uptake characteristics of these complexes under conditions simulating the therapeutic conditions.

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MAJOR FINDINGS: Ion exchange separations of platinum species in aqueous media, at concentrations similar to those used clinically, can be made quantitative with respect to each of the species separated and provide excellent data for kinetic studies of the complex species transformation reactions. Together with other data on the behavior of chemical species in the presence of protein in solution, they are providing the basis for a systematic study of this complex system.

PROPOSED COURSE: The principle work on method development and reaction kinetics, thus far, has been with CDDP. Further studies are planned to establish the effects of difference reaction conditions, including the effects of nucleophiles, on the rate of complex transformations and protein binding. Studies with CDDP in vitro and in vivo are planned together with studies of some of the second of the the second generation platinum drugs in clinical use or in toxicologic evaluation studies. Preliminary experiments suggest the applicability of the analytical techniques already developed is generally valid with minor modifications required in some cases.

PUBLICATIONS:

"Uptake of platinum compounds in Human Tumors. In Vitro Study"
Hecquet, B., LeRoy, A., Lefebvre, J-l., Adenis, L.,
Bulletin du Cancer (In Press) 1986

"Determination of cis-diamminedichloroplatinum II in human plasma using 'Ion Pair' chromatography with electrochemical detection"
Parsons, P. J., LeRoy, A. F.
Accepted for publication - Journal of Chromatography - Biomedical Applications, 1986

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RS 10216-02 BEI

108

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Enzyme Purification - Calmodulin

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)
A.F. LeRoy Ph.D., BEIB/DRS
R. Berger, Ph.D., LTD, NHLBI

COOPERATING UNITS (if any)

LAB/BRANCH
Biomedical Engineering and Instrumentation

SECTION
Analytical Methods

INSTITUTE AND LOCATION
DRS, National Institutes of Health, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Validity of studies characterizing and measuring properties of calmodulin depend on having quantifiable measures of purity of the enzyme preparation and the proportion of calcium it contains and requires the availability of extremely pure reagents, buffer, and solvents and contamination free labware.

Analytical methods have been developed for quantitaiton of picogram quantities of calcium in highly purified preparations of calmodulin and in the reagents and solvents needed to make the preparations. Preliminary analyses show that the needed reagents and solvents for making ultra-pure enzyme preparations can be freed to satisfactory levels from such ubiquitous contaminants as calcium using techniques developed in our laboratory.

PHS 6040 (Rev. 1/84) GPO 914-418

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OBJECTIVES: Develop techniques for quantitation of calcium at picogram levels in samples containing microgram quantities of enzyme and develop supply of reagents and solvents necessary for making enzyme preparations of the desired purity in the quantity needed for NIH studies.

MAJOR FINDINGS: Analytical Methods have been developed for quantitation of picogram quantities of calcium in highly purified preparations containing microgram quantities of calmodulin. Validity of method has been confirmed through analysis of appropriate NBS Standard Reference Materials. Preliminary analyses show that the needed reagents, buffers, and solvents can be freed from such common contaminants as calcium using techniques developed in our laboratory to concentration levels that are satisfactory and can be made in the necessary quantity for present laboratory needs.

PROPOSED COURSE: Develop larger scale system for production of purified reagents, buffers, and solvents in the quantities needed as related research project activity increases need. Develop suitable facilities as anti-contamination requirements become more stringent.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RS 10218-02 BEI

110

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Analytical Electron Microscopy of Adrenal Chromaffin Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

R.D. Leapman	Visiting Scientist	BEIB, DRS
Others:		
R.L. Ornberg	Biophysicist	LCBG, NIDDK
H. Pollard	Chief	LCBG, NIDDK

COOPERATING UNITS (If any)
Computer Systems Laboratory, Division of Computer Reserach and Technology, NIH (K.E.Gorlen)

LAB/BRANCH
Biomedical Engineering and Instrumentation

SECTION
Electron Beam Imaging and Microspectroscopy Group

INSTITUTE AND LOCATION
DRS, National Institutes of Health, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.7	0.7	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The elemental composition in various compartments of the bovine adrenal chromaffin cell was investigated by a combination of energy-dispersive x-ray spectroscopy and electron energy loss spectroscopy. Cryosections of quick-frozen cultured cells were transferred at low temperature (-170 C) into the Hitachi H700H analytical electron microscope and the samples were carefully dehydrated in situ. Spectra were recorded from: (1) secretory granules, (2) cytoplasm, (3) nuclei (4) mitochondria and (5) extracellular space. X-ray spectra were quantitated by means of specially prepared standards containing known amounts of protein and ions. Concentrations accurate to ± 5% or 10% could be established for most of the major elements. Electron energy loss spectra were analyzed with software developed in BEIB to determine ratios of nitrogen-to-phosphorus.

Experiments have been performed to investigate the effect of pH on catecholamine secretion and how this is correlated with changes in ionic distributions in the cell. Measurements have also been made on isolated secretory granules.

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OBJECTIVES: To investigate how secretion of catecholamines occurs in the adrenal chromaffin cell and how the secretory granules are formed. These processes depend strongly on the composition of the granules which has not yet been measured in complete cells.

METHODS EMPLOYED: Ultrathin cryosections of quick-frozen cells were transferred at low temperature into the analytical electron microscope. X-ray spectra were recorded with a Tracor Northen TN5500 multichannel analyzer and silicon detector. Electron energy loss spectra were recorded with a Gatan 607 magnetic sector spectrometer. X-ray spectra were analyzed by means of a multiple least squares fitting procedure for all the X-ray peaks in the spectrum. Standards also prepared by rapid freezing were used to obtain weight factors for the concentrations. Simultaneous acquistion of energy loss spectra as well as the dark field elastic image was achieved.

MAJOR FINDINGS: Determination of the chlorine concentrations inside and outside the secretory granules gave an estimated membrane potential consistent with other work. The concentration of potassium of ca 170 mmole/Kg dry weight was much higher than expected on the basis of studies on isolated granules and suggests that potassium may be strongly bound.

Calcium concentration was high in the secretory granules (ca 15 mmole/Kg dry wt.) but was very low (ca 1mmole/Kg dry wt.) in the mitochondria and higher (ca 5mmole/Kg dry wt.) in the cytoplasm containing ER. This is consistant with other work which suggests that mitochondria do not regulate cytoplasmic calcium. Preliminary data show that ammonia treated cells have elevated calcium in the granules and elevated chlorine in the cytoplasm suggesting that pH dependent ion exchanges are occurring across the granule membrane.

SIGNIFICANCE: In order to understand how secretion of catecholamines occurs and how these compounds are packaged, it is important to obtain information about the distribution of ions in the various compartments of the cell.

PROPOSED COURSE: It is planned to make further measurements on resting and stimulated cells and to treat cells so as to change the membrane potential and pH. Experiments are also planned to determine concentrations of catecholamines directly perhaps using fluorine-labelling.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RS 10219-02 BEI

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Quantitative Elemental Mapping of Rapidly-frozen Neural Tissue

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

R. D. Leapman	Visiting Scientist	BEIB
Others:		
S.B. Andrews	Special Expert	LN, NINCDS
T.S. Reese	Chief	LN, NINCDS
C.E. Fiori	Physical Scientist	BEIB, DRS
C.R. Swyt	Physical Scientist	BEIB, DRS

COOPERATING UNITS (if any)
Computer Systems Laboratory, Division of Computer Research and Technology, NIH (K. Gorlen)

LAB/BRANCH
Biomedical Engineering and Instrumentation

SECTION
Electron Beam Imaging and Microspectroscopy Group

INSTITUTE AND LOCATION
DRS, National Institutes of Health, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The elemental composition of the presynaptic and postsynaptic nerve terminals of rapidly-frozen synapses of the mouse cerebellar molecular layer was determined using electron probe x-ray microanalysis and elemental imaging by characteristic x-rays. Quantitative electron probe analysis of synaptic vesicle clusters and intraterminal mitochondria indicated that their calcium concentrations were 0.4 ± 0.1 and 1.2 ± 0.2 mmol/l respectively. Similar experiments were carried out on cerebellar slices rapidly frozen after incubation in vitro. The distribution of K and Ca in presynaptic terminals of resting and depolarized slices were qualitatively and quantitatively similar to those in fresh slices. Results suggest that the vesicles have a limited role in calcium storage and release. In contrast measurement of calcium in the postsynaptic region of the cerebellar molecular layer show evidence for calcium sequestration in a smooth membrane cistern of the derdritic process called the "spine".

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OBJECTIVES: To obtain elemental distributions in rapidly frozen preparations of mouse cerebellum and to relate these distributions to the ultrastructure of the synaptic contacts.

METHODS EMPLOYED: Rapidly frozen ultrathin cryosections of mouse cerebellar cortex were studied in a Hitachi H700H analytical electron microscope equipped with a TN5500 multichannel analyzer and silicon detector. X-ray spectra were analyzed by means of a multiple least squares approach to fit all peaks in the spectrum.

MAJOR FINDINGS: In both resting and stimulated preparations, clusters of synaptic vesicles as well as single axonal mitochondria contained approximately 1mmol/l of Ca and normal levels (approx 100 mmol/l) of internal K. In these same preparations the postsynaptic regions of the dendritic processes (spines) contained cisterns with approximately 3 to 5 mmol/l of Ca.

SIGNIFICANCE: Ca-handling in cerebellar presynaptic terminals is characterized by relatively subtle ion movements. In the post-synaptic regions there is a non-mitochondrial site that accumulates readily detectable amounts of extracellular Ca in response to membrane depolarization.

PROPOSED COURSE: To improve to spatial resolution of microanalysis by making use of the field emission-source STEM that is on order in BEIB.

PUBLICATIONS: Andrews, S.B., Leapman, R.D., Landis, D.M.D. and Reese, T.S.: The distribution of calcium and potassium in presynaptic nerve terminals from cerebellar cortex, Proc. Nat. Acad. Sci. 1986 (in press).

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RS 10221-02 BEI

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Elemental Imaging of Alveolar Macrophages

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)
C.R. Swyt, Physical Scientist BEIB, DRS
C.E. Fiori, Physical Scientist, BEIB, DRS
R.D. Leapman, Visiting Scientist, BEIB, DRS
W. Rom, Senior Fellow, PB, NHLBI

COOPERATING UNITS (if any)

LAB/BRANCH
Biomedical Engineering and Instrumentation

SECTION
Electron Beam Imaging and Microspectroscopy Group

INSTITUTE AND LOCATION
DRS, National Institutes of Health, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Refractile particles are observed by light microscopy (LM) in alveolar lavaged macrophages from patients exhibiting clinical symptoms of asbestosis, silicosis and coal-miner's emphysema. However the elemental composition and location of these particles in the ultrastructure of the macrophages cannot be determined by LM. Elemental x-ray images obtained in the CAMECA MBX microprobe from large fields of whole air-dried macrophages have enabled investigators to correlate particle composition with disease. High resolution images of individual macrophages in ultrathin sections of epon embeded preparations obtained in the Hitachi H700H analytical electron microscope utilizing bright field (BF), energy dispersive x-ray (EDS) and electron energy loss (EELS) signals have revealed both elemental composition and ultrastructural location of the particles.

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OBJECTIVES: The objectives of the collaboration were to determine the composition of refractile particles observed by light microscopy in the alveolar lavaged macrophages of patients exhibiting clinical symptoms of asbestosis, silicosis, and coal-miner's emphysema, and to correlate element with clinical condition and ultrastructure.

METHODS EMPLOYED: Aliquots of the order of 1 ml of H20 washed fixed cell suspensions were dispersed by aerosal spray onto spectroscopically pure carbon substrates. Not fewer than 50 cells of each preparation were imaged in the CAMECA MBX microprobe using the secondary electron signal and the wavelength dispersive x-ray signals from two selected elements simultaneously. The elements tabulated in this study were Ca, Si, Mg and Fe. Preparations of cells from both normal volunteer controls and clinically diagnosed patients were imaged.

Ultra-thin sections of conventionally prepared epon embeded suspensions were imaged in the Hitachi H700H analytical electron microscope using signals from a Tracor Northen side looking energy dispersive x-ray detector, a magnetic sector EELS spectrometer and a conventional bright field detector. Images of the distribution of C, Fe, Si, and Mg were produced using the EELS and EDS signals.

The x-ray signals from selected elements were acquired concurrently. All data were acquired under control of imaging software written by CSL/DCRT and running on the PDP 11/60 computer.

SIGNIFICANCE: Elemental analysis of particulates observed in alveolar lavaged macrophages could reduce ambiguity of cause of pulmonary disease diagnosed from clinical symptoms.

PROPOSED COURSE: Analysis of data will be completed. Images of biopsied alveolar tissue will be acquired in the analytical microscope to determine distribution of particles in the lung tissue itself.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RS 10222-02 BE1

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Elemental Distributions in Central Nervous System Tissues

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

C.R. Swyt Physical Scientist BEI3/DRS
R.M. Garruto Senior Biologist CNSS/NINCDS
C.E. Fiori Physical Scientist BEIB/DRS

COOPERATING UNITS (if any)
CNSS/NINCDS

LAB/BRANCH
Biomedical Engineering and Instrumentation

SECTION
Electron Beam Imaging and Microspectroscopy Group

INSTITUTE AND LOCATION
DRS, National Institutes of Health, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1	1	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The occurrence of neurofibrillary tangles and plaques is common to a number of central nervous system disorders including Guamanian amyotrophic lateral sclerosis (ALS), parkinsonism with dementia (PD), and Alzheimer's disease. These NFTs and plaques contain nonessential metals and elevated levels of biological elements. Fixed and frozen thick sections of hippocampus and frontal cortex of human brain from subjects exhibiting symptoms of these diseases have been examined in the CAMECA MBX microprobe. Wave length dispersive x-ray spectrometry (WDS) performed at selected areas has provided both spectra and digital images showing the distribution of elements of interest in the specimens.

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OBJECTIVES: The objeccive of this collaboration is to obtain both x-ray spectra and digital x-ray images of the distribution of calcium, aluminum and other implicated elements in sections of brain from control cases and cases showing pathological symptoms of these central nervous system diseases, and to establish correlation of elevated concentrations with morphology from the gross to ultrastructural levels.

METHODS EMPLOYED: 20 micron thick sections of fixed and frozen sections of hippocampus and frontal cortex are cut and mounted on spectroscopically pure carbon substrates. The pathology of the specimen has been characterized by light microscopy (LM), frequently on adjacent sections. Sections are examined in the secondary electron imaging mode (SEI) of the CAMECA MBX microprobe and the areas to be imaged are selected by characteristic morphology. Under computer control, the SEI signal and, simultaneously, the back-scattered electron signal (BSE) and two characteristic x-ray WDS signals are collected from a regular array of up to 512x512 analysis points in a field of up to 1mmx1mm. These arrays of data are displayed as digital images. The characteristic x-ray images reveal the distribution of the two elements selected and can be quantified. The SEI and BSE images provide correlation of elemental deposition with the characteristic morphology.

MAJOR FINDINGS: Intraneuronal accumulations of calcium and aluminum and silicon have been confirmed in Guamian ALS, PD and Alzheimer's disease specimens.

PROPOSED COURSE: Work will continue on the disease specimens mentioned by the methods described. Specimens for analysis will also be provided from subjects with other central nervous system disorders such as Down's syndrome, Creutzfeldt-Jakob disease and kuru. Higher resolution work will be pursued on the Cameca microprobe, and analysis of ultrathin embeded and cryo-sections and isolated neurofilaments will be performed on the Hitachi H700H analytical electron microscope.

PUBLICATIONS:

Duckett, S., Galle, P., Fiori, C.E.: Electron probe microanalysis of normal and pathological neural tissue with wavelength dispersive x-ray spectrometry Metal Ions In Neurology and Psychiatry, Alan R. Liss Pub, New York pp 369-398, 1985.

Fiori, C.E., Swyt, C.R.: Elemental x-ray microanalysis of matter by means of focused electron probes. In Elving, P.J. (Ed.): Treatise on Chemistry 2nd Ed., John Wiley and Sons, NY, 1986, 8: 311-358.

Garruto, R.M., Swyt, C.R., Fiori, C.E., Yanagihara, R., and Gajdusak, D.C.: Intraneuronal deposition of calcium and aluminum in amyotrophic lateral sclerosis of Guam. Lancet, 2, pp 1353-1354, 1985.

Garruto, R.M., Swyt, C.R., Fiori, C.E., and Gajdusek, D.C.: Intraneuronal colocalization of silicon with calcium and aluminum in amyotropic lateral sclerosis and parkinsonism with dementia of Guam. New England Journal of Medicine, 315, pp. 711-712, 1986.

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Fiori, C.E.: Confusion of osmium M-zeta with aluminum K-alpha lines in biological x-ray microanalysis. Lancet, 1986.

Fiori, C.E., Swyt, C.R., and Ellis, J.R.: A critique of the continuum normalization method used for biological x-ray microanalysis. In Joy, D.C., Romig, A.D., and Goldstein, J.I. (Eds.): Principles of Analytical Electron Microscopy, Plenum Press, NY, 1986, pp. 413-443.

Fiori, C.E., Leapman, R.D., and Gorlen, K.E.: Computer-aided compositional mapping in scanning electron column devices. In Armstron, J.T. (Ed.): Microbeam Analysis - 1985, San Francisco Press, 1985, pp. 219-226.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RS 10225-02 BEI

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Processing of High Resolution Electron Micrographs

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Michael Unser	Visiting Fellow	BEIB	DRS
Benes Trus	Research Chemist	CSL	DCRT
Alasdair C. Steven	Visiting Scientist	LPB	NIADDK

COOPERATING UNITS (If any)

LAB/BRANCH
Biomedical Engineering and Instrumentation

SECTION
Office of the Chief

INSTITUTE AND LOCATION
CRS, National Institutes of Health, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

High resolution micrographs are often of poor quality, due to various distortions and especially to a very low signal-to-noise ratio. Visual quality can be improved significantly by using correlation-averaging techniques to combine several observations of similar subjects.

We had previously developed methods for translational and rotational alignment of different views of ostensibly identical specimens. These techniques have been extended for the compensation of spatial deformations in slightly distorted periodic structures.

Multivariate statistical techniques have been implemented for the analysis and comparison of sets of images. Quantitative approaches have been developed for the automated removal of anomalous views and the assessment of spatial properties of the resulting averaged image (e.g., rotational symmetry and resolution).

These methods have been applied successfully to structure determination and quantitative assessment of Herpes Simplex type II Virus capsomers, T7 Virus, and skeletal muscle filament images.

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OBJECTIVES There are currently four main objectives in this research: (1) The determination of appropriate statistical models (noise and various distortions) for high resolution micrographs, (2) the study of algorithms for the alignment of specimen views using simple (translation/rotation) or more complex geometrical transformations; (3) the decision procedure which determines the classification of different views as well as the detection of "bad" samples; (4) the assessment of the quality of the result obtained by averaging (e.g., signal-to-noise improvement, resolution).

METHODS EMPLOYED: Several programs have been developed in FORTRAN 77 and added to an existing library on CSL's image processing facility (VAX 780). Standard supervised and unsupervised classification algorithms (minimum distance, K-means, hierarchical clustering, etc...) have been implemented. More specific methods were designed for the automated detection of "bad" subjects (OMO algorithm) and the quantitative assessment of specimen properties using variance ratios (comparison of sub-groups of particles, determination of rotational symmetry, and spatial resolution). The restoration of slightly distorted periodic structures is based on a geometrical transformation of the data using bi-dimensional polynomials.

MAJOR FINDINGS: The procedure that compensates for geometrical deformation (unwarping) in quasi-periodic structures allowed a significant restoration of high electron micrographs of myofilament lattices in vetrebrate muscle.

The use of quantitative methods turned out to be extremely useful. By systematically removing inconsistent views, the OMO algorithm allowed an improvement of the quality of the globally averaged Herpes capsomer, T7 virus, and muscle filament images. The application of statistical criteria led to the quantitative determination of a rotational 6-fold symmetry and a resolution of 2.5nm for the averaged Herpes capsomer, and a 9.0nm resolution for the restored muscle filaments.

SIGNIFICANCE: The acquired scientific expertise and the developed software should allow other scientists, primarily at NIH, to use these image processing and computer reconstruction techniques to determine or understand a specimen's structure. Furthermore, the use of statistical methods provides quantitative results and facilitates decision making.

PROPOSED COURSE: The comparison of lower dimensionality data representations (Principal components, correspondence analysis) and the evaluation of the various quantitative approaches which have been developed are still under investigation. We are planning to design more flexible iterative methods for the restoration of quasi-periodic structures. An improved restoration of muscle filament images would also necessitate the estimation and compensation of the microscope transfer function, as well as the effect of tilt. In addition, we will study reconstruction techniques from projected data for three dimensional structure determination.

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PUBLICATIONS

Unser M., Steven, A., and Trus, B.: Odd men out: a quantitative objective procedure for identifying anomalous members of a set of noisy images of ostensibly identical specimens, Ultramicroscopy,in press.

Unser, M., Trus, B., and Eden, M.: Unwarping of slightly distorted periodic structures using bidimensional polynomial representations, Signal Processing, in press.

Steven, A.C., Trus, B.L., Unser, M., McDowall, A.W., Dubochet, J., and Podolsky, R.J.: Myofilament lattice of vertebrate skeletal muscle visualized by correlation averaging of frozen hydrated thin sections, In Bailey, G.W., (Ed.), Proc. 44th Annual Meeting of the Electron Microscopy Society of America, San Francisco Press, 1986, pp. 108-109.

Trus, B.L., Steven, A.C., Unser, M., McDowall, Dubochet, J., and Podolsky, R.J.: Correlation averaging techniques to rectify disordered myofilament lattices visualized in frozen hydrated thin sections of vertebrate muscle, Proc. XIth International Congress on Election Microscopy, Aug. 31-Sept. 7, Kyoto, Japan, 1986, pp. 3103-3104.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RS 10227-01 BEI

PERIOD COVERED: October 1, 1985 to September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
In Vivo Evaluation of Pulsed Intra-Arterial Infusions

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

D.R. Shook,	Biomedical Engineer	BEIB, DRS
S. Saris,	Staff Neurosurgeon	SNB, NINCDS

COOPERATING UNITS (if any)
Diagnostic Radiology, CC; Surgical Neurology, NINCDS;
Veterinary Resources Branch, DRS

LAB/BRANCH
Biomedical Engineering and Instrumentation Branch

SECTION
Mechanical Engineering Section

INSTITUTE AND LOCATION
DRS, National Institutes of Health, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.05	0.05	

CHECK APPROPRIATE BOX(ES)

- [X] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Diastolically phased pulsed intra-arterial infusions have been shown to be an effective means to produce concentration uniformity in downstream branches of a model of the cerebral circulation. The technique is being evaluated in the cerebral circulation of fully anesthetized Rhesus monkeys by the infusion of radio-isotope labelled iodoantipyrine into the internal carotid artery. The resulting tissue concentration is determined by quantification of autoradiographs.

Initial results show high uniformity of tissue concentration, indicating that the deleterious effects of infusate streaming can be alleviated.

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OBJECTIVES: To perform an in vivo evaluation of diastolically phased, pulsed intra-arterial infusions and study the effects of phased pulsing on infusate concentration uniformity in perfused tissues.

METHODS: The pulsed infusion of radio-isotope is made through a 30 ga. needle placed in the internal carotid artery. The infusion continues for approx. 30 sec and the needle withdrawn. The uniformity of cortical tissue uptake is determined by quantification of autoradiographs indicating isotope concentration at regular spatial intervals. The pulse control parameters, i.e., average infusion rate, pulse infusion rate, pulse width, beat/pulse ratio, and delay are available on a modified angiographic injector (Medrad, Inc, Pittsburgh, PA) coupled to a fast response solenoid valve.

MAJOR FINDINGS: Initial results correlate well with in vitro studies, Visual observation of autoradiographs reveal very uniform density along the cortical strip. Autoradiographs are being analyzed quantitatively for comparison to well mixed, intravenous data.

SIGNIFICANCE: In Vitro studies of phased, pulsed infusions have been most encouraging and the technique is well suited for the clinic without changes in established treatment protocols. Verification of these results in animals and analysis of perfused tissue concentration uniformity will establish this technique as perhaps the only viable one to alleviate streaming in low infusion rate clinical protocols.

PROPOSED COURSE: Analyze autoradiographs of pulse-infused tissue. Compare with intravenously infused (non-pulsed) data. Optimize the pulsed infusion parameters for various protocols to maximize mixing efficacy.

Related Project Number:

Z01-RS-10001-18 BEIB
Z01-RS-10206-02 BEIB (Pulsed Intra-Arterial Infusions)

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RS 10228-01 BEI

124

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Automated Medication Dispenser

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)
C.P. Mudd, Biomedical Engineer, ACES, BEIB, DRS
H. Tipton, Engineering Technician, ACES, BEIB, DRS
Russell T. Wall, Anesthesiologist, CC, ANES

COOPERATING UNITS (if any)
ANES, CC

LAB/BRANCH
Biomedical Engineering and Instrumentation Branch

SECTION
ACES

INSTITUTE AND LOCATION
DRS, National Institutes of Health, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	

CHECK APPROPRIATE BOX(ES)

- [X] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The automated medication dispenser consists of a timing system to release medication at prescribed intervals of 2, 3, or 4 hours and an electronic security system to prevent unauthorized access to the medication. One or two pills can be dispensed per dose interval to provide up to 24 pills/day for 3 days without reloading. When the dispenser is loaded, a two digit patient identification number, 00 to 99, is set on two thumbwheel switches located in the pill compartment. After loading the medication, the dispenser can be locked. With the dispenser in operation, the medication is released into a holding cell at the prescribed intervals. When the medication reaches the holding cell, a opto-electronic detector senses its presence and stops the timing process. At this time, the patient must perform two acts to obtain the medication: (1) enter the two digit identification number into the dispenser via two pushbutton switches next to the release bar, and (2) press the release bar. If the patient entered identification number agrees with internal preset number, the release bar is activated and will release the medication if it is present in the holding cell. If the patient entered number is incorrect or there is no medication in the holding cell, the release bar remains disabled and will not function. Any attempt on the release bar resets the number entered by the patient to 00. All attempts on the release bar are recorded for use in adjusting the dose rate.

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OBJECTIVES: The dispenser was designed to accomplish two objectives: (1) free the nursing staff from the repetitive tasks of delivering medication and responding to patient requests for medication before the scheduled time and, (2) create an atmosphere in which the patient assumes some responsibility for self-medication and thus feels more in control of his or her situation.

SIGNIFICANCE: Similar devices exist for the dispensing of fluid medication and have performed well in accomplishing the above mentioned objectives. This solid medication dispenser should enjoy the same utility and success.

PROPOSED COURSE: One proto-type has been constructed and is now undergoing evaluation in the Clinical Center.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RS 10229 BEI

126

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
All Tantalum Stopped Flow Differential Micro-Calorimeter

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

C.P. Mudd,	Biomedical Engineer	ACES, BEIB, DRS
R. Berger,	Chemist	LTD, NHLBI

COOPERATING UNITS (if any)
LTD, NHLBI

LAB/BRANCH
Biomedical Engineering and Instrumentation Branch

SECTION
ACES

INSTITUTE AND LOCATION
DRS, National Institutes of Health, Bethesda, MD, 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Based upon the sensor design techniques recently used in batch micro-calorimeters, we have devised an all tantalum stopped flow calorimeter with significantly greater sensitivity and faster response times. The tantalum flow assembly consists of two inlet tubes and one outlet tube which are electron-beam welded to a tantalum mixer. An array of small, fast response thermopiles are located on opposite sides of the outlet tube. The outlet tube contains a volume of 350 micro-liters. A 0.1 mm stainless wire is also attached to the outlet tube for use in calibration. Two such assemblies are used in this differential instrument. The sensing portion of the tubing is mounted in a large heat sink and kept at the core or operating temperature of the calorimeter. As the tubes enter and leave the heat sink, they are routed together so that thermal artifacts propagating down the tubes will appear as common-mode and not differential signals. The resulting instrument has a sensitivity of 1 joule/volt*second and a 10 to 90% step response of 48 seconds.

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OBJECTIVES: The objective was to design and fabricate a stopped flow micro-calorimeter with the sensitivity and response characteristics of the earlier batch heat conduction calorimeters. The stopped flow technique adds the important advantage of fast cycling time and computer controlled mixing.

SIGNIFICANCE: If successful, this approach will yield the same information as that obtained in the heat conduction batch calorimeters but at a rate 20 to 50 times faster with the added advantage of computer control.

PROPOSED COURSE: A proto-type has been constructed and is currently undergoing evaluation. The sensitivity and response time are as expected but differential flow artifacts are present which limit the useful sensitivity. Efforts are underway to identify the source(s) of these artifacts and correct them.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RS 10230-01 BEI

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Television Tracking of Beating Heart Cells in-vitro

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

W. Schuette,	Chief	ACES, BEIB, DRS, NIH
J. Parrillo,	Chief	CCMD, CC, NIH
C. Burch		CCMD, CC, NIH

COOPERATING UNITS (if any)
DRS, BEIB
CCMD, CC

LAB/BRANCH
Biomedical Engineering and Instrumentation Branch

SECTION
Applied Clinical Engineering

INSTITUTE AND LOCATION
DRS, National Institutes of Health, Bethesda, MD, 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	0.6	0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A Television Tracking System has been developed to measure the motion of beating Heart Cells in-vitro. The performance of these cells is used to monitor the presence of a circulating Myocardial Depressant Substance in Humans with septic shock. Beating myocardial cells were placed in a petri dish on the microscope stage at an inverted microscope and imaged by a television camera. Motion voltages were developed from the resultant television video by the tracking system.

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PUBLICATIONS:

Parrillo, J.E., Burch, C.S., Natanson, J.H., Natanson, C., Schuette, W.H: "A Circulating Myocardial Depressant Substance in Humans with Septic Shock", The Journal of Clinical Investigation 76:1539-1533, (1985).

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RS 10231-01 BEI

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Instrumentation Development for Fluorescence Spectroscopy

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

J.L. White,	Electronic Engineer	EEES, BEIB, DRS
J.R. Knutson,	Sr. Staff Fellow	LTD, NHLBI

COOPERATING UNITS (If any)
LTD, NHLBI

LAB/BRANCH
Biomedical Engineering and Instrumentation Branch

SECTION
Electrical and Electronic Engineering

INSTITUTE AND LOCATION
DRS, National Institutes of Health, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Automatic control of a time-resolved fluorescence spectrophotometer was developed to enable the rapid collection of fluorescence spectra. Several peripherals of the laser based system are controlled by a Hewlett-Packard Series 1000 mini-computer, including the acquisition, transfer, and storage of data, the selection of samples, and the automatic positioning of monochromators for wavelength selection. The control of these peripherals allows data to be collected more quickly than is possible with a manual system, with little or no operator intervention, and also allows maximum flexibility in the design and enhancement of experiments.

Specific experiments that incorporate computer control and acquisition include alternating between sample and reference lamp data acquisition at fixed wavelengths to achieve fluorescence decay curves, and collecting time-resolved fluorescence data from a sample while rapidly scanning through a series of wavelengths. Because modular programming was used, new experiments can be easily configured.

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OBJECTIVES: The purpose of this technique is to provide a rapid, automatic means of collecting time-resolved fluorescence data using an existing spectrophotometer. Objectives include little or no operator intervention, rapid customization of experiments, and the ability to incorporate future system enhancements.

METHODS EMPLOYED: A Hewlett-Packard Series 1000 mini-computer was programmed in FORTRAN to control existing peripheral instruments. These include a multi-channel analyzer for data collection and a stepper motor controller for monochromator positioning and sample selection. Existing programs were modularized and expanded, and new programs, including automatic instrument initialization and calibration, customization of experimental parameters, sample and wavelength selection, data acquisition, data transfer and storage, and error processing, were designed. The modules were written to be easily combined in a variety of configurations, which allows for the rapid design of control for new experiments.

SIGNIFICANCE: Computer control of the fluorescence spectroscopy system offers several advantages over manual control. The static and dynamic structures of biological macromolecules and membranes can be investigated using fluorescence spectroscopy as a tool. Because of the complex nature of the fluorescent spectra and decays from biological materials, an instrument is required that can recover multiple, mixed signals from the emissions that these systems display. Automation of the instrument greatly speeds the collection of data so that extensive data analysis is possible in a reasonable time period.

PROPOSED COURSE: The programs that control experiments will be tied to an existing package of data analysis programs so that the data collection/data analysis link will also be automated. Instrument enhancements will include protective circuitry for the laser crystal, monitoring of the actual excitation flux, the ability to scan excitation, and improvement of the photodiode timing reference.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RS 10232-01 BEI

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Hardware Expansion for Flow Cytometer

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

C.C. Gibson, Electronics Engineer, EEES, BEIB, DRS
J.A. Dvorak, Dr. LPD, NIAID

COOPERATING UNITS (if any)
LPD, NIAID
ACES, BEIB, DRS
EUII, BEIB, DRS

LAB/BRANCH
Biomedical Engineering and Instrumentation Branch

SECTION
Electrical and Electronics Engineering

INSTITUTE AND LOCATION
DRS, National Institutes of Health, Bethesda MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	0.3	0.7

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The Flow-Cytometer in LPD has had extensive modifications made to it in two distinct areas. This lab has been responsible for the enhancement of the computer system and the system electronics. The computer was upgraded from a DEC PDP-8 to a DEC PDP11/40 and then to a DEC PDP11/34 with a 67Mb disk system and a 1600 Bpi tape drive. Along with the hardware changes, numerous software changes are planned to ease the operation of the Flow-Cytometer in the future.

The forward angle scatter detector has been replaced along with the Laser and most of the Flouresence optics (ACES). Also a Coulter Volume circuit has been added and many changes have been made to the interface electronics to increase the sensitivity of the instrument.

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OBJECTIVES: The purpose of this project is to provide a flow cytometer that is easy to use and that is more sensitive than any commercial flow cytometer.

METHODS EMPLOYED: A DEC (Digital Equipment Corp.) PDP11/34 computer was programmed in FORTRAN to collect and analyze flow cytometer data in real time. Specialized hardware was fabricated to interface the computer to the flow cytometer and every effort was made to maximize the sensitivity of the instrument.

SIGNIFICANCE: The Flow-Cytometer in LPD is currently the most sensitive flow cytometer on the NIH campus, allowing the researcher to study small parisites and yeasts that, until now, have been impossible to study.

PROPOSED COURSE: In the future the disk units will be upgraded, the instrument will be modified to provide greater resolution, and the software will be modified to provide automatic disk selection and file numbering along with better display routines.

BOOK:

Author: Dvorak, J.A., Gibson, C.C., Maekelt, A.: A Bibliography on Chagas Disease. PAHO Publication, 1985, RD 2411.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RS 10233-01 BEI

PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Computer System for Analysis of Monkey Vocalizations

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

C.C. Gibson, Electronics Engineer, EEES, BEIB, DRS
Dr. D. Symmes, LCE, NICHD

COOPERATING UNITS (if any)

LCE, NICHHD
EUII, BEIB, DRS

LAB/BRANCH

Biomedical Engineering and Instrumentation Branch

SECTION

Electrical and Electronic Engineering

INSTITUTE AND LOCATION

DRS, National Institutes of Health, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.2	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A computer system has been assembled to analyze monkey vocalizations and display the results in the traditional format of waterfall displays.

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OBJECTIVES: The objective of this project is to provide a rapid means of analyzing the vocalizations of monkeys and to display the results to the researcher in a form that is meaningful.

METHODS EMPLOYED: A DEC (Digital Equipment Corp.) PDP11/73 computer was employed and additional hardware was added to provide 100KHz A/D digitization of the signal and an array processor to process the data very rapidly. The programming is done in PASCAL and an effort has been made to provide a menu driven system that is easy to use.

SIGNIFICANCE: Understanding the vocal patterns and learning of "speech" by monkeys will help the researcher to understand the learning patterns of human children.

PROPOSED COURSE: Software will be written to utilize the high speed hardware and a DMA link will be made to a commercial speech analysis system thus providing two methods of digitizing the data.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RS 10234-01 BEI

136

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Computer Aided Tracking of Myosin Coated Beads on Actin Filaments

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

C.C. Gibson,	Electronics Engineer	EEES, BEIB, DRS
D.Sellers,		LMC, IR, NHLBI

COOPERATING UNITS (if any)
LMC, IR, NHLBI
EUII, BEIB, DRS

LAB/BRANCH
Biomedical Engineering and Instrumentation Branch

SECTION
Electrical and Electronics Engineering

INSTITUTE AND LOCATION
DRS, National Institutes of Health, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.3	0.2	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A system using a microcomputer with a graphics board, mouse, timer, and video board is being put together with a color TV camera and a VCR to track Myosin coated beads over time as they interact with Actin. The programming is being done in complied BASIC and Pascal to collect and display the data.

GPO 814-814

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OBJECTIVES: Tracking the Myosin coated beads *in vitro* allows a large number of experiments to be performed on myosin-base motility.

METHODS EMPLOYED: The assay is based on the concept that myosin beads will interact with actin filaments only when the beads are properly oriented to produce force. Those myosin beads in improper orientations do not bind to actin and have no effect on motility. Thus, a structure will occur in a direction defined entirely by the polarity of the actin filaments. This concept has been put into practice by coating latex spheres with myosin and applying then to a polar array of actin filaments from the fresh water alga *Nitella axillaris*. Under proper conditions, the myosin spheres move unidirectionally on the organized actin filament arrary.

SIGNIFICANCE: The assay described here is well suited to study the effects of modification of myosin on the speed with which it moves. Filaments formed by dilution or dialysis can be added directly to the beads, or individual myosin molecules can be attached in high salt to particles by way of antibodies that recognize the myosin cell.

PROPOSED COURSE: As soon as all the hardware is delivered the software will be checked out and the unit will be delivered. The software will be re-written in PASCAL to provide higher speed and more versatility.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 10235-01 BEI

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Filtration of Cholesterol in vivo from Human Subjects

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

C.C. Gibson,	Electronics Engineer,	EEES, BEIB, DRS
Dr. H. Klein		BB, CC

COOPERATING UNITS (if any)
BB, CC
EUII, BEIB, DRS

LAB/BRANCH
Biomedical Engineering and Instrumentation Branch

SECTION
Electrical Electronic Engineering

INSTITUTE AND LOCATION
DRS, National Institutes of Health, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.2	0.3

CHECK APPROPRIATE BOX(ES)

☑ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Some human subjects have extremely high levels of cholesterol and an experimental protocol has been devised to filter out large amounts of cholesterol using a commercial cell separator followed by filtration of the plasma and re-injection of the plasma into the subject.

PHS 6040 (Rev. 1/84) GPO 914-818

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OBJECTIVES: Removal of large amounts of cholestrol from human subjects without complicated, manual methods now used.

METHODS EMPLOYED: The plasma will be separated from the whole blood using a commercial cell separater and the red cells returned to the patient. After the plasma is separated it is heated to 42.0 deg. C and pumped through an experimental filter to remove the cholesterol, cooled to 37.0 deg. C and returned to the patient.

SIGNIFICANCE: If this procedure works, the cholesterol level in certain human subjects can be reduced much further than by present methods and many more subjects could be treated.

PROPOSED COURSE: Purchase or build the equipment to heat the plasma to 42 deg. C and to cool the plasma to 37.0 deg. C with a doublely redundant safety circuit to prevent overheating of the plasma or not cooling enough before return to the subject.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RS 10236-01 BEI

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Instrumentation for Various Electron Microscopes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

C.C. Gibson	Electronics Engineer	EEES, BEIB, DRS
C.E. Fiori	Physicist	OC, BEIB, DRS

COOPERATING UNITS (if any)
EUII, BEIB, DRS
CSL, DCRT

LAB/BRANCH
Biomedical Engineering and Instrumentation Branch

SECTION
Electrical and Electronic Engineering

INSTITUTE AND LOCATION
DRS, National Institutes of Health, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.3	0.1	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Planning is underway to control the Cameca Microprobe with an IBM PC-XT equipped with a number of add in peripheral cards to control the wavelength spectrometers, collect the various analog and digital signals, control the stage movement, transfer the collected data to the PDP11/60 for display and analysis, and provide miscellaneous functions such as nitrogen filling, beam blanking, and contrast control.

PHS 6040 (Rev. 1/84) GPO 914-018

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OBJECTIVES: To provide an inexpensive, convenient method for controlling the Cameca microprobe for image acquisition without some of the present problems.

METHODS EMPLOYED: An IBM PC-AT or clone will be purchased with the additional boards necessary to interface the Cameca microprobe to the computer. All programming will be done in house in either "C" or PASCAL to control the microprobe.

SIGNIFICANCE: An inexpensive method to control the Cameca microprobe without dependence on others for programming will enable the researchers to tailor the system to their needs in a timely fashion.

PROPOSED COURSE:

(1) Purchase the necessary Hardware and compliers
(2) Write drivers for the individual pieces of hardware
(3) Develop control and applications software to control the microprobe and modify existing programs to analyse the data.
(4) Use the instrument to collect and analyse data.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RS 10237-01 BEI

PERIOD COVERED: October 1, 1985 to September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.): Text to Digital Character Conversion

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

J.R. Ellis	Expert	OC, BEIB, DRS
H. Marshall		OC, OD
M. Brandenberg		OC, OD
J. Gruber		EU1, SES, BEIB, DRS

COOPERATING UNITS (If any): OC, OD

LAB/BRANCH: Biomedical Engineering and Instrumentation Branch

SECTION: Office of the Chief

INSTITUTE AND LOCATION: DRS, National Institutes of Health

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.15	.10	.05

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects
  - ☐ (a1) Minors
  - ☐ (a2) Interviews
- ☐ (b) Human tissues
- ☒ (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The NIH Office of Communications desired to form a computer readable list of NIH alumni, current and former affiliations, and addresses for the the NIH Centennial celebration. In general, the digital tape records of personnel and affiliations are no longer available. Many relevant printed and typed documents, such as the series of NIH Scientific Directories, are available. This organization wished to read such records and use the results for mailing lists, and other record keeping for this reunion.

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OBJECTIVES: Conversion of many kinds of text records to computer usable form.

METHODS EMPLOYED: The office of Communications of the Office of the Director had been told of the Kurzweil reading machine in the BEIB SERP. We proposed to used this machine for ASCII character output, initially with a terminal and WYLBUR (or another available host machine), and perhaps later with a Personal Computer, to test its viability for reading their lists.

SIGNIFICANCE: There are many records of importance to various organizations available in printed or typewritten form, which they would like to be able to search and process digitally. Convenient, efficient transformation from human-readable form to machine-readable form would make many projects feasible which are not now in that category.

MAJOR FINDINGS: We managed to obtain ASCII output from this machine, although this had not been done previously here. The Kurzweil Model 1II Reading Machine was not adequate for the task of reading the mimographed lists or the printed Scientific Directories.

Because this particular commercial machine was obsolete, more modern reading machines were tried at both NIH and at the National Library of Medicine (NLM). The results were similar. In all cases, the typewritten list, which was of low quality for a human reader, had an unacceptably large error rate when read by machine. The printed material in the Scientific Directories simply had too frequent changes of font and size for the automated modes to function effectively.

We conclude that data should be kept in digital form on some medium wherever possible. If typewritten or printed data are to be used in a commercially available reading machine, the quality of such data should be high, and the number of different fonts, sizes etc. should be kept to an absolute minimum.

PROPOSED COURSE: Interested parties should be advised that the current state-of-the art in commercial reading machines does not come anywhere close to the flexibility of a fairly well-trained and motivated human operator. Data entry on a local machine, certainly a personal computer, is generally going to be more economically feasible and satisfactory. In many cases, use of a terminal connected to a central utility, such as WYLBUR, will also be more satisfactory.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RS 10238-01 BEI

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Automation of BEIB Information Processing Functions

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)
J.R. Ellis, Expert, OC, BEIB, DRS
A.G. Stringer, Administrative Officer, OC, BEIB, DRS
H.S. Eden, Deputy Chief, OC, BEIB, DRS
C.Martin, Management Analyst MAO, DRS

COOPERATING UNITS (if any)
MAO, DRS
CSL, DCRT

LAB/BRANCH
Biomedical Engineering and Instrumentation Branch

SECTION
Office of the Chief

INSTITUTE AND LOCATION
DRS, National Institutes of Health, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	2.0	0.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The computational and accounting resources of the branch are often inadequate to the demands placed on them. The objective of this project is three-fold. The first is to determine the needs of the branch. Second, we need to identify and obtain appropriate resources from the modern world of computer science and data processing. Third, there is a requirement to match resources in a fast-moving scenario and changing user demands that cannot be specified in advance.

PHS 6040 (Rev. 1/84) GPO 914-818

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OBJECTIVES: To provide in-house, modifiable and upgradable computational capabilities for administrative accounting and budgeting, scientific word processing, and reporting in a manner which minimizes duplicate or multiple data entry.

This must be done so that the output is compatible with existing and projected interfaces established by DRS, DCRT, DFM, and other units within NIH with which the branch must interact. Any system also must meet the constraints established by these organizations, especially DCRT.

METHODS EMPLOYED: We have attempted to determine the needs of the branch through interviews, meetings with personnel, and study of forms used for various purposes. Several hardware and software options have been considered, and their advantages and disadvantages weighed. Since there are several personal computers already in the branch, and the facilities of DCRT are available, use of the IBM mainframe and personal computers has been given some study. The suitability of choices made by the Personal Workstation Office of DCRT (PWO/DCRT) for our purposes has been considered, and we have called upon the expertise of the Computer Systems Laboratory (CSL/DCRT) in personal computers and networking.

Scientific Equipment Services has installed a local area network and is using a number of IBM PCs for accounting functions. In addition to its current function, this project gives us valuable insight into the problems to be expected with a branch-wide system.

We have installed an ethernet which is compatible with and connected to DCRT's ethernet. This provides reliable, high-speed, easy-to-use access to our machines, and access to many machines in buildings 12 and 12A. Locally, our network connections include a 3Com disk and printer server to link the IBM Pcs, four Bridge communication servers with fourteen ports each for terminals and terminal emulation, and four Hewlett-Packard Laser Printers. Further, we have two Apple MacIntoshes and an Apple Laserwriter on a local AppleNet.

SIGNIFICANCE: Successful completion of this project or substantial portions thereof should make the document (paperwork) flow smoother and faster, improve the efficiency of forms preparation and reporting procedures dramatically, and allow fiscal and status accounting to be vastly easier and more effective.

MAJOR FINDINGS: There is a well-known need for improvement of the "BEIB System" of accounting provided by DCRT/DMB. The SERP System administered by DRS/MAO is better suited to the current tasks, primarily because it is newer, but is still regarded as inadequate to the future needs of this fast-growing program.

There is a less well recognized need for administrative reporting and scientific text preparation. Some staff members of the branch find WYLBUR adequate; most do not.

The vast majority of tasks performed by branch personnel involve relatively small data bases and documents which can be prepared independently and then need to be compiled in various ways into coherent whole items. Thus, a distributed system is regarded as better suited to meeting our needs than a centralized one.

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Finally, there are needs which are seldom articulated because they are not regarded as being within the capabilities of existing systems.

NIH has standardized on the IMB PC family for many of its functions. This standard must be met for many external purposes, especially administrative.

PROPOSED COURSE: We propose to meet the needs of the branch primarily by using a network of personal computers with compatible file structures.

A VAX-11/750 has been acquired by the branch, and should be used for scientific computations and simulations requiring its substantial capabilities.

The Apple MacIntosh, with its icon-based interface, seems to be better suited to scientific word processing and to use by infrequent users. We feel that MacIntoshes can coexist with the IBM standard set by PWO/DCRT. Although only a few programs currently allow direct transfer of data between the MacIntosh and MS-DOS, we anticipate that more will be available in the future.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RS 10240-01 BEI

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Clinical Measurement of Microvascular Blood in Sickle Cell Patients

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)
E.C. Walker, Mechanical Engineer, A.C.E., BEIB
G.P. Rodgers, Clinical Associate, LCB, NIDDK

COOPERATING UNITS (if any)

LAB/BRANCH
Biomedical Engineering and Instrumentation Branch

SECTION
Applied Clinical Engineering

INSTITUTE AND LOCATION
DRS, National Institutes of Health, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	

CHECK APPROPRIATE BOX(ES)

- [X] (a) Human subjects
  - [X] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Current viewpoints assume that manifestations of sickle cell disease result from the obstruction of capillary networks by abnormal erythrocytes during deoxygenation. Using a commercially available Laser Doppler Velocimeter (Med Pacific LD 5000) we propose to monitor non-invasively cutaneous blood flow in stable sickle cell patients under various conditions of steady state and perturbed flow. Additionally, since red cell deformability is a key factor in determining capillary flow we will monitor in vitro filtration rates of cells under various conditions.

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OBJECTIVE: To characterize the degree and pattern of cutaneous blood flow in sickle cell patients. In particular, to evaluate the efficacy of treatment modalities such as hyperoxia. A further objective is to evaluate the usefulness of the LD5000 in monitoring cutaneous blood flow.

METHODS: The Med Pacific LD5000 Laser Doppler Velocimeter is being used to monitor capillary blood flow. The LD5000 is currently interfaced to a tektronix computer system for on-line data collection. Various sites (primarily the forearm) will be studied.

The necessary instrumentation will be designed to perturb the local blood flow. Data analysis will be dependent upon observed findings. One interesting flow pattern thus far observed in sickle cell patients is oscillatory flow. Time and frequency domain analysis on this flow pattern will be performed. Sickle cell data will be compared to data from normal patients using standard statistical methods.

SIGNIFICANCE: These results should indicate the capability of the LD5000 in monitoring cutaneous blood flow and therefore its usefulness as a clinical tool. Our findings should also demonstrate the effectiveness of hyperoxia treatment on improving local blood flow. Furthermore these results should show the flow profile of compromised blood cells while traversing the microvasculature and suggest improved methods of treatment.

PROPOSED COURSE: Plans for this fiscal year are to complete a preliminary patient survey using the LD5000/computer system, finalize the algorithms for data analysis, and complete the design of instrumentation for perturbing local flow. Hyperoxia studies should also be initiated.

PUBLICATIONS:

Acquage, C., Walker, E.C., Schecter, A.N.: The development of a filtration system for evaluating flow characteristics of erythrocytes. Micro. vasc. Res. (in press, fall 1986)

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RS 10241-01 BEI

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Cisplatin Kinetics

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)
P.J. Parsons Visiting Fellow, BEIB, DRS
P.F. Morrison, Physical Scientist, BEIB, DRS

COOPERATING UNITS (if any)

LAB/BRANCH
Biomedical Engineering and Instrumentation Branch

SECTION
Chemical Engineering

INSTITUTE AND LOCATION
DRS, National Institutes of Health, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Experimental and theoretical studies on the chemistry of cisplatin (DDP) have been initiated with the goal of characterizing the reaction kinetics of the drug in vitro. This knowledge should lead to an identification of the principal reactive species and pathways, with attendant implications for the design of drug protocols.

Refinement of the separation and detection techniques required for the investigation has been undertaken. An improved high-performance liquid chromatography and hanging mercury drop electrode technique has been used to follow the fate of DDP and its major hydrolysis products in various aqueous media. Peaks corresponding to the two metabolites have been identified by using off-line fraction collection with platinum determination by atomic absorption methods; kinetic analysis of these platinum-containing peaks has confirmed their identities. The results of kinetic studies are in remarkably good agreement with results of other researchers, but they leave many unanswered questions pertaining to the nature of the reaction mechanism.

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OBJECTIVES: Refinement of an analytical method for the separation of platinum species derived from the antitumor drug cisplatin (DDP). Improvement of the detection limit for platinum species using a hanging mercury drop electrode. To use the improved method to study the rate of change of DDP, and other platinum species, in vitro, with whole plasma and plasma fractions; moreover, to fit the kinetic data to a suitable mathematic model.

METHODS EMPLOYED: A high-performance liquid chromatography technique has been developed and refined for the separation of platinum species. This is achieved by using ion-pair reagents which chemically modify a reversed-phase column. Of the various kinds of columns and ion-pair reagents investigated, a 15 cm C_8 column modified with 5mM heptanesulphonic acid was found to be superior for separating DDP from its cationic hydrolysis products. Detection was achieved by using a hanging mercury drop electrode, held at -250 mV vs. Ag/AgCl. Whole human plasma was subjected to centrigugal ultrafiltration to produce a range of plasma fractions. Atomic absorption methods were used to aid identification of Pt-containing peaks.

MAJOR FINDINGS: An improvement in the detection limit for the major hydrolysis product was achieved by adjusting the electrode potential during the analysis such that during elution of DDP, it is -250 mV, but for the hydrolysis product, it is switched to -1.0 V. This improvement provided evidence for the presence of this hydrolysis product in plasma ultrafiltrate, some 8 hours after incubation in vitro, even with a chloride ion concentration of 100 mM. In water at 37 C, the rate of appearance of the hydrolysis product closely paraileled the disappearance of DDP.

SIGNIFICANCE: The quest for a better understanding of the mechanism by which cisplatin operates is limited by the analytical techniques available for the determination of platinum species al low levels. The development of such an analytical method has permitted an in depth study of the reaction mechanism of DDP with various nucleophiles. This knowledge should lead to an identification of the principal reactive species and pathways, with attendant implications for the design of drug protocols.

PROPOSED COURSE: Further experiments are planned in which the fate of DDP, and its hydrolysis products, will be followed in an aqueous solution containing a number of potential nucleophiles endogenous to human plasma ultrafiltrate. Further kinetic analysis of this data will help elucidate the reaction mechanism.

PUBLICATIONS

Parsons, P.J. Morrison, P.F., LeRoy, A.F., "Determination of Platinum-Containing Drugs in Human Plasma by Liquid Chromatography with Reductive Electrochemical Detection", submitted to J. Chromatography, 1986.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RS 10242-01 BEI

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Automated Method for the Detection of Animal Circling

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)
A. Markowitz, Electronic Engineer, ACES, BEIB, DRS
W. Schuette, Chief,ACES, BEIB, DRS
B. Chidakel, Electronic Tech, BEIB, DRS
K. Bankiewicz, Physician, NINCDS
T. Tedder, Mechanical Tech, BEIB, DRS

COOPERATING UNITS (if any)

LAB/BRANCH
Biomedical Engineering and Instrumentation Branch

SECTION
Applied Clinical Engineering

INSTITUTE AND LOCATION
DRS, National Institutes of Health, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.0	0.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Parkinson's disease is chemically induced by infusion of MPTP into the right internal carotid artery of an animal. The infusion produces injury to one side of the brain, causing Parkinson symptoms and spontaneous motor activity of circling toward the injured side. Treatment with drugs (apomorphine or L-DOPA/ carbidopa) or brain implants causes circling towards the intact side.

An automated method to measure this circling as a function has been developed. The rotation of the animal is plotted as a bar graph as function of time. The previous method of monitoring animals by means of television was both time consuming and expensive, especially with multiple animal experiments.

The system consists of back-pack-mounted infra-red (IR) emitting transmitters, cage mounted IR sensors, specialized electronics and a microcomputer system capable of monitoring many animals simultaneously.

Each IR transmitter consists of an oscillator pulsing four IR light emitting diodes (LED), mounted on a 9 volt transistor radio battery and inserted in a jacket worn by the animal. A radiation pattern of 180 degrees is achieved by the transmitter. An IR phototransistor is the receiver, and it is mounted in the center of each cage wall. The output of each detector is amplified by a high pass amplifier, full wave rectified, processed by a slow time constant low pass filter and then presented to a 32 channel A/D converter located in a Zenith Z-100 PC computer system.

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The data from each cage are processed in the computer to determine the current orientation of the animal. Each time the orientation changes to an adjacent side, a fractional counter is incremented or decremented by one count, depending on the direction of animal circling. A plus four on the counter would indicate a complete clockwise rotation and the fractional counter is then reset to zero. A minus four would indicate a complete counterclockwise rotation and the counter is again reset to zero. The total of clockwise and counter clockwise rotations are written to a disk file and the computer screen every five minutes. The disk files may be recovered and plotted as a bar graph with each bar representing a five minute period.

The system enables continuous monitoring of many animals over an extended period of time and is able to tell quickly and effectively the success of any drug or brain implant.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RS 10243-01 BEI

PERIOD COVERED: October 1, 1985 to September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.): Using Acyclovir to Prevent UVB Light Reactivation of Herpes Simplex

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

R. Corsey Electronic Engineer BEIB, DRS
S. Straus Section Chief NIAID
J. Rooney Research Associate NIDR
A. Notkins Research Associate NIDR

COOPERATING UNITS (if any): Nursing Department, Clinical Center

LAB/BRANCH: Biomedical Engineering and Instrumentation Branch

SECTION: Applied Clinical Engineering

INSTITUTE AND LOCATION: DRS, National Institutes of Health, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.5	0.5

CHECK APPROPRIATE BOX(ES)

- [X] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Ultraviolet light (UVB) has been demonstrated to induce reactivation of Herpes Simplex Virus in non-genital areas in which prior recurrences had been observed. The purpose of this study is to evaluate the ability of acyclovir to suppress the induction of recurrent Herpes Simplex Virus infection by UVB light. Patients will be randomized on a cycle of radiation to receive oral acyclovir or placebo pills for six days in a double-blind study. Each cycle will consist of four challenge irradiations with four miminal erythematous doses of UVB light each. Each patient will be followed for documentation of recurrent lesions induced by UVB light or spontaneously occuring lesions throughout the course of the study. Acyclovir has been shown to have significant effects on pain, lesion crusting, lesion healing and viral sheading when administered intravenously. It has also been shown to have significant efficacy in relief of pain, itching and viral sheading time when topically administered. Oral as opposed to intravenous or topical acyclovir will be used exclusively in this study. An area of skin which is near but not overlapping sites previously involved with herpetic recurrence will be selected. A template will be utilized to isolate four 1 cm in diameter areas of skin. A special apparatus which can hold four fluorescent tubes at a distance of 25.4 cm from the target site will be utilized. The fluorescent tubes will have output primarily of UVB (290-320 nm) with a reduced output of UVA (320-400 nm) average caucasian skin pigmentation would call for about four minutes exposure with increased melanin requiring more exposure time.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RS 10244-01 BEI

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Exogenous Insulin to Treat Anorectic Weight Losing Cancer Patients

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

R. Corsey Electronic Engineer, BEIB, DRS
J. Norton Section Chief, NCI
K. Kern Research Associate NCI

COOPERATING UNITS (if any)
Nutrition Department Clinical Center, NIH
Nursing Department Clinical Center, NIH

LAB/BRANCH
Biomedical Engineering and Instrumentation Branch

SECTION
Applied Clinical Engineering

INSTITUTE AND LOCATION
DRS, National Institutes of Health, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.5	0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Weight loss is a common problem in patients with cancer, affecting their quality of life and their ability to respond to therapy. Total parenteral nutrition has not been able to reverse the body depletion phenomenon to the point where oncologist question the efficacy of its use in the cancer patient. On the other hand, insulin has been shown to improve food intake, nitrogen balance and body weight in wasting-state rats without stimulating tumor growth. Insulin treatment has also been shown to increase body cell mass, the fundamental structural component of animals. In rat studies, this improvement has led to improved survival following tumor resection. This study will evaluate 20 patients who have lost at least 10% of normal body weight, have biopsy proven cancer and are in an anorectic state. The patients also must have a functioning gastro-intestinal tract. The following questions will be investigated.

1. Can low-dose exogenous insulin improve body weight, cell mass, food intake, nitrogen balance and body catabolism of an anorectic patient with cancer?
2. Can exogenous insulin accomplish the above without developing dangerously low blood sugar levels?

A whole body counter will be used to measure the K40 level of each patient. This measurement relates directly to the body cell mass. Resting energy expenditure will be determined and the metabolic kitchen will be utilized for a balance study.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RS 10245-01 BEI

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Positions Emission Tomography in the Study of Extremity Soft Tissue Sarcomas.

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

R. Corsey	Electronic Engineer	BEIB, DRS
J. Norton	Section Chief	NCI
A. Chang	Research Associate	NCI

COOPERATING UNITS (if any)
Nutrition Department, Clinical Center, NIH
Department of Nuclear Medicine, Clinical Center, NIH
Laboratory of Pathology, National Cancer Institute, NCI

LAB/BRANCH
Biomedical Engineering and Instrumentation

SECTION
Applied Clinical Engineering

INSTITUTE AND LOCATION
DRS, National Institutes of Health, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.5	0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Positron Emission Tomography (PET) can be used to measure glucose utilization in human tumors. Since rapidly growing poorly differentiated neoplasms have higher rates of glycolysis than slow growing well differentiated neoplasms, PET scanning may be used to categorize the degree of malignancy. It has already been used to predict survival time in glioma patients and it has been used to image glucose utilization in extremity soft-tissue sarcomas. Since no study has correlated glucose utilization rate in extremity sarcomas with histologic grade or clinical outcome, the present study will attempt to answer the following questions:

1. Can PET scan results be used to estimate glucose utilization rates reproducibly?
2. Do PET scan results of glucose utilization rates correlate with histopathology as read in the Department of Pathology?
3. What is the glucose 6-phosphatase level of fresh human tumor after resection of extremity sarcomas?
4. What is the fresh glucose 6-phosphatase activity of human muscle from extremity sarcoma specimens?

Patients will be studied for resting energy expenditure by the method of indirect calorimetry. This will help gather information about any distant metabolic effects produced by soft-tissue sarcomas resulting in abnormal glucose metabolism.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RS 10246-01 BEI

PERIOD COVERED: October 1, 1985 to September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Acousto-Optically Controlled Light Source for Microscopy

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	P.D. Smith	Physicist	BEIB, DRS
Others	K.G. Spring		LKE, NHLBI

COOPERATING UNITS (If any)

LAB/BRANCH: Biomedical Engineering and Instrumentation Branch

SECTION: Electronic and Electrical Engineering Section

INSTITUTE AND LOCATION: DRS, National Institutes of Health, Bethesda, Md. 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.25	.25	

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects
  - ☐ (a1) Minors
  - ☐ (a2) Interviews
- ☐ (b) Human tissues
- ☒ (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

An optical system, featuring an acousto-optic element, was designed as an illumination source for a microscope. This arrangement provides significant advantages over conventional light sources. First, the wavelength of illumination can be readily changed; second, the intensity of the illumination can be adjusted to a constant level; third, both the wavelength and intensity changes can be accomplished at rates up to several MHz by signals originating in computer software.

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OBJECTIVE: To provide a variable wavelength excitation source for fluorescence microscopy. These excitation wavelengths were to be selected from a cw argon ion laser with access to each wavelength of excitation being achieved at rates in excess of video framing rates.

METHODS EMPLOYED: A Lexel model 95 argon ion laser operates in the cw mode with six lines in the blue-green wavelength region of the spectrum, viz 457, 476, 488, 496, 501, and 514nm. These lines do not have the same intensity with the major energy being in the 488 and 514 nm lines. The output beam has a typical beam diameter ($1/e^2$ points) of 0.9mm with an output beam divergence (full angle) of 0.4mrad.
An optical system coupled this output through an acousto-optic device into a Leitz fluorescence microscope. The acousto-optic device is an IntraAction model ADM-40 deflector which is capable of achieving 100 spot resolution. To select the 496 and 501nm lines as discreet wavelengths, an input beam divergence of less than 0.05mrad is required since this angular separation represents a 5nm wavelength separation in the output of the acousto-optic deflector operating at the Bragg angle. Thus, in the horizontal deflection plane, the input optical system to the acousto-optic element must provide a reduction in the laser beam divergence and at the same time expand the beam to fill the aperture of the acousto-optic element. This is conveniently accomplished by a 12mm focal length, cylindrical lens - 25cm focal length, biconvex achromatic lens telescope combination. In the vertical plane this combination provides an additional function of concentrating the laser beam in the acoustic field of the acousto-optic element. This permits high efficiency deflection of the laser beam into the 1st Bragg order.

The deflected laser beams, propagating at six different directions in space, are collected by an identical 25cm achromatic lens and brought to a focus with a 100cm lens. At the focal plane of this lens, a 20u slit is positioned with permits selection of the spatially separated independent argon lines. After a specific wavelength passes through the slit the light is collimated and introduced into the illumination port of the microscope.

Wavelength selection through the 20u slit is accomplished by adjusting the frequency of the acoustic signal applied to the acousto-optic element. The IntraAction driver electronics permits this frequency to be adjusted 10MHz on either side of the 40MHz center frequency. This is more than adequate to separate the argon ion laser lines. Intensity control is provided by adjusting the acoustical power delivered to the acousto-optic element which determines the efficiency of the Bragg diffraction into the 1st order. Both the frequency and intensity controls are capable of being driven externally from computer software originating commands. The response of the acousto-optic element to these commands is determined by the propagation time of the acoustic signal across the acousto-optic element. Typically rates in excess of 1MHz are realized.

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MAJOR FINDINGS: The acousto-optic element and the optical arrangement resolved the 457, 477, 488, and 514nm lines, but there was overlap of the 496 and 501nm lines. The optical system only reduced the beam divergence of the input beam to the acousto-optic element to 0.04mrad, which is marginal for separating this pair of wavelengths. For the particular task for which is optical system was designed, separating these wavelengths was not necessary and no attempt was made to further reduce the input beam divergence. The intensity of the other four argon laser lines were adjusted to give equal intensity of illumination. The particular settings of the acoustic frequency and power for each line were optimized for each wavelength line and subsequently sent electronically by hardware implemented software commands from a laboratory computer. These settings were found to be sub-optimal until the acousto-optic element had thoroughly warmed up for 30 minutes, but thereafter remained stable.

SIGNIFICANCE: This method of providing illumination to a microscope has several advantages over the conventional methods of illumination when rapid change from one wavelength to another is required. In such situations, the choice is limited to approaches such as spinning filter wheels or adjusting a dispersive element, for example a prism, either in a monochromator or in a laser. In all cases, access times are limited to a mechanical adjustment, random selection is not possible, and intensity control is difficult. The acousto-optic element overcomes these problems and is able to provide MHz rates of transition from one setting to the next. In the particular context of the apparatus for which the acousto-optic element optical system was designed, these access times were tailored to the video framing rates, since an ultra-sensitive image intensifier - video camera system was used as the detector of the fluorescence signal originating in the biological preparation.

PROPOSED COURSE: Extension of the principle of acousto-optic selection and modulation to white light sources will be investigated.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RS 10247-01 BEI

PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Effect of Mitral Valve Replacement On Left Ventricular Geometry

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Thomas L. Talbot, M.S. ACES BEIB DRS
Robert J. March, M.D. SB NHLBI
Richard E. Clark, M.D. SB NHLBI

COOPERATING UNITS (If any)

Surgery Branch, NHLBI

LAB/BRANCH

Biomedical Engineering and Instrumentation Branch

SECTION

Applied Clinical Engineering Section

INSTITUTE AND LOCATION

DRS, National Institutes of Health, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☑ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The correction of chronic mitral insufficiency by conventional mitral valve replacement carries a higher postoperative morbidity and mortality than operation for most other valvular lesions. Clinical studies have shown that valvuloplasty performed to correct mitral insufficiency is associated with a lower morbidity and mortality than with valve replacement. It is suggested that the maintenance of the mitral apparatus (chordae tendinae) prevents enlargement of end-diastolic and end-systolic dimensions. By utilizing each ventricle as its own control, the effect of chordal severence after valve replacement on regional and global geometry was examined. Two groups of animals (chronic and acute) were compared.

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METHODS EMPLOYED: Eight normal sheep underwent insertion of a bioprosthesis, along with wire chordal snares without excision of any portion of the mitral apparatus. Left ventricular mechanics were then studied before and after complete division of the mitral chordae. The animals were instrumented with ultrasonic dimension transducers to measure the base-apex, anterior-posterior, and septal-free wall diameters, as well as lateral wall thickness of the LV. High fidelity micromanometers measured ventricular pressures. Using cardiopulmonary bypass and hypothermic cardioplegic arrest, a 25 mm pericardial valve was inserted in the mitral annulus with preservation of the native mitral apparatus. After each animal had been weaned from bypass and allowed one hour recovery, pressure and dimension data were collected at matched heart rates before and after severing the mitral chordae with transventricular snares. Analog signals were digitized and analyzed with interactive software. Ellipsoidal shell theory was utilized to calculate left ventricular volume and wall stress.

MAJOR FINDINGS: Following division of the mitral chordae, the end-diastolic wall stress increased 48% with concomitant increases in LV end-diastolic pressure of 30% and end-diastolic volume of 14%. The increase in end-diastolic volume was accounted for by an increase in the base-apex (EDA) left ventricular axis without a demonstrable significant change in the anterior-posterior or septal-free wall end-diastolic dimensions. Despite an increase in preload, stroke volume did not increase significantly. However, there was decreased systolic excursion in the base-apex dimension without a demonstrable significant change in the excursion of the other two LV axes.

The second group of animals were each given a 4mm defect in one of the mitral leaflets and returned to pasture for approximately six months. This group upon examination revealed severe mitral regurgitation with concomitant enlargement of the left ventricle. The chronic group of animals was studied in the same manner described above. This group showed similar increases in end diastolic volume and wall stress, however this group had an increase in stroke volume and ejection fraction. Despite these observations which might suggest increased ventricular function, systolic analysis revealed a marked decrease in the maximum systolic pressure which the ventricle produced, and a significant decrease in the maximum rate of change of the left ventriculor pressure.

Present analysis is focused on explaining some of these parodoxes which the preliminary analysis has brought to light.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RS 10248-01 BEI

PERIOD COVERED

October 1, 1985 - September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Intra-arterial Optical Evaluation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institution affiliation)

PI:	P.D. Smith	Physicist	BEIB	DRS
Others	R.F. Bonner	Biophysicist	BEIB	DRS
	R. Balaban	Physical Scientist	LKE	NHLBI
	M.B. Leon	Cardiologist	CB	NHLBI
	D. Lu	Clinical Assoc.	CB	NHLBI
	R.L. Bowman	Chief	LTD	NHLBI

COOPERATING UNITS (if any)

NHLBI

LAB/BRANCH

Biomedical Engineering and Instrumentation

SECTION

Electrical and Electronic Engineering Section

INSTITUTE AND LOCATION

DRS, National Institutes of Health, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	

CHECK APPROPRIATE BOX(ES)

- [X] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Evaluation of the intra-arterial wall surface is necessary for control of surgical procedures envisioned using laser ablation of tissue. Angioscopy inside vessels produces clear observation of the target sites provided adequate irrigation of saline solution is maintained. Precise quantitative location of the target is difficult. In addition, size constraints may limit angioscopy's usefulness in the coronary vessels.

Preliminary fluorescent observation of human atheromatic and normal aorta specimens was performed using a fluorescence microscope with 450-490nm excitation. Fluorescence spectra collected using a multi-channel analyzer indicated clear intensity differences between the normal and atheromatic tissue. These differences were reduced and eliminated by ablation of the atheroma by either an argon laser (514nm) or a xenon chloride (308) excimer laser.

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OBJECTIVE: To provide a method of identifying atheromatic lesions or other sites within the vasculature and to permit control of laser devices being used in surgical procedures.

METHODS EMPLOYED: Olympus 1.8mm diameter, 50cm long and 2.5mm diameter (with 0.5mm port), 80cm long angioscopes were coupled using a specially designed lens attachment to a Sony DXC1850 color camera. The output was recorded on U-Matic video tape and displayed on a high resolution color monitor. The field of observation was illuminated by an Olympus fiber-optic light source, and manual control of illumination was provided to ensure optimal lighting. Introductory catheters were fabricated from latex to allow free passage of the angioscope and to permit adequate flow of irrigating fluid to clear the field. Pressure cuff bags were found to be suitable for pressurizing the sterile plastic pack containers of 0.9% normal saline used for irrigation.

Fluorescence measurements were made using a Leitz microscope using epi-illumination of the tissue sample. A Leitz filter cube passing 450-490nm was used for excitation and the light emanating from the sample was focussed by the objective through the filter cube on to the slit of a Jobin-Yvon monochromator. At the output focal plane of the monochromator was placed a SIT vidicon detector whose scanning could be controlled to permit target integration. Standard video processing techniques were available. Laser ablation of the tissue was accomplished by either 5W of an argon laser focussed through a 10cm focal length lens on to the tissue for 1-2 seconds or by 15mJ per pulse of 308nm XeCl excimer radiation brought to a focus by a 5cm focal length lens. This latter permitted successive etching of the target to be achieved in a superficial manner.

MAJOR FINDINGS: Both angioscopes produced excellent clarity and were easy to use in the vessels producing little or no trauma. The introductory catheters were adequate and permitted, through a Y-connector, passage of both the angioscope through one arm and the saline flushing solution through the other arm. The major limitation in use of the angioscope into small vessels, this required large pressures in the driving bag, and a point was reached when sufficient irrigation could not be achieved. Quantitatively, it was found impossible to determine how far away the tip of the angioscope was from the lesion identified on the screen of the monitor. This is a common problem of transposing the tubular view to a flat screen display. If the tube diameter or some other feature is known, then it is possible to calibrate the screen in terms of distance from the tip of the angioscope. In the vascular circulation the vessel diameter is not in general known. To incorporate the laser fiber optic into the angioscope poses additional problems. Angioscopes are not currently available with fibers capable of transmitting either near-uv or infra-red wavelengths which seem to be the most suitable for ablative micro-surgery. Thus either special angioscopes of the appropriate materials or angioscopes incorporating a lumen of adequate diameter must be available for ablative surgery. In the latter case, the overall size of the complete device becomes too large for general use.

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An alternative approach is to use an automated technique to determine the type of tissue directly in front of the fiber. The fluorescence properties of normal and atheromatic aorta tissue were found to exhibit different fluorescent signals when excited with light of 450-490nm. This fluorescent signal had a spectral peak at 540nm with a weaker shoulder at 580nm, and the ratio of the peak at 540nm of normal to diseased aorta was 2.3. This ratio was the largest observed for similar experiments carried out with excitation in the uv (340-380nm) and in the green (530-560)nm. No specific chromophore could be identified with the spectral profile. This difference in peak intensity disappeared when the atheroma was irradiated by either the argon or excimer lasers, and in the latter case, a gradual increase in the fluorescence signal emanating from the formed crater could he observed following successive hits of the atheroma. This suggests that the atheroma acts as a filter or scatterer of the incident or emitted light from the chromophore embedded in the tissue.

An oscillating mirror, which could be synchronized to the laser firing frequency, was developed to permit introduction of the fluorescence excitation light into the same fiber that is used for conducting the laser energy. Possible use of a beam splitter is not favored since the fluorescent signal is extremely weak and the contamination of the detection system by the flashlamp or plasma glow excitations of the two laser systems is highly likely. In addition, in the excimer case, damage to the beam splitter is probable due to the very high power levels involved with this irradiation.

SIGNIFICANCE: The ability to be able to determine the target for the laser is desirable, since the laser radiation is indiscrimate in that it can cut through normal arterial wall with the same degree of efficiency as diseased tissue. The fluorescent signal can be incorporated into a feedback mechanism to determine whether the laser can be fired safely or not. The use of angioscopy is not limited to coronary arteries, and if size concerns limit its usefulness there, the ability to examine larger vessels within the vasculature is of great utility.

PROPOSED COURSE: To incorporate the fluorescent signal detection into a fiber delivery system, and to further study the signal to try and determine it origin. In addition, the method is lacking in it ability to assess what is the normal level of fluorescence, and a study will be conducted to decide whether other excitation wavelengths are more appropriate to allow a ratio-based scheme to select the type of tissue being targetted.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RS 10249-01 BEI

PERIOD COVERED: October 1, 1985 to September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.): Kinetics of Folate Metabolism

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.F. Morrison,	Physical Scientist	BEIB, DRS
C.J. Allegra,	Physician	CP, NCI

COOPERATING UNITS (if any): Clinical Pharmacology Branch, NCI

LAB/BRANCH: Biomedical Engineering and Instrumentation

SECTION: Chemical Engineering

INSTITUTE AND LOCATION: DRS, National Institutes of Health, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects
  - ☐ (a1) Minors
  - ☐ (a2) Interviews
- ☐ (b) Human tissues
- ☒ (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Over the last decade, research has shown that the antifolate effects of the drug, methotrexate (MTX), are mediated by its polyglutamate metabolites. The polyglutamates are known to be responsible, directly or indirectly, for inhibition of both thymidylate and purine synthesis. Ultimately we are interested in formulating a biochemical kinetic model useful for exploring various hypotheses of purine inhibition by MTX, but before this is possible, the kinetics of MTX cellular transport and of its polyglutamation must be described.

Accordingly, the polyglutamation kinetics of methotrexate (MTX) in MCF-7 human breast cancer cells have been formulated mathematically. The model accounts for glutamation and hydrolysis kinetics up through the pentaglutamate level, increased synthesis of dihydrofolate reductase following exposure to drug, reversible tight-binding to reductase, and membrane transport of all the drug polyglutamates. The glutamation, hydrolysis, and efflux parameters have been determined from fits to experimental MTX polyglutamate uptake and efflux data. The preferred substrate for folypolyglutamyl synthase in the intact cell has been shown to be MTX diglutamate, on average being 2 to 3 times as reactive as either the parent drug or the triglutamate. Hydrolysis rate constants exhibit no clear trend with chain-length because of the large uncertainty of each parameter estimate. However, the efflux of MTX polyglutamates from MCF-7 cells does show a trend with chain-length, decreasing with increasing length as expected. The model also shows quantitative agreement with the fraction of MTX polyglutamates found still to be bound to reductase in MCF-7 cells following 24 hrs of efflux.

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OBJECTIVES: To formulate a kinetic model of the glutamation, hydrolysis, and membrane transport characteristics of the methotrexate polyglutamates, thus developing a means for estimating the amounts of highly active drug species formed during arbitrary dose and exposure time.

METHODS EMPLOYED: A set of ordinary differential equations was formulated describing the glutamation, hydrolysis, and transport of MTX polyglutamates up through the pentaglutamate level. Using kinetic results from isolated enzyme studies, it was argued that both glutamation and hydrolysis could be represented by linear reaction terms, provided the drug concentration to which cells are exposed does not much exceed 10 M. Influx transport of the parent drug was described by Michealis Menten kinetics while efflux was left linear. Transport of the polyglutamate derivatives was taken to be linear, with the diglutamate represented by one rate constant and the tri to pentaglutamate group by another. The MTX polyglutamates were taken to bind tightly and reversibly to dihydrofolate reductase in competition with the normal dihydrofolate substrate. Based on experimental observation, dihydrofolate concentration itself was taken to be constant but greatly elevated above its normal value. The induction of dihydrofolate reductase in the presence of MTX was taken into account by representing it as an increase in enzyme synthesis rate.

The nine parameters of this model were evaluated by fitting the solutions of the non-linear differential equations to polyglutamate concentrations measured after various lengths of exposure of MCF-7 human breast cancer cells to drug-containing or drug-free media (influx and efflux experiments). Regression routines of the Levenberg-Marquardt and Newton method types were employed to obtain best mean estimates and variance-covariance matrices.

MAJOR FINDINGS: The preferred substrate for folylpolyglutamyl synthase in intact cells was shown to be MTX diglutamate with greater than 85% confidence. The magnitude of hydrolase constants was determined to be in the range of .04 hr^{-1} to .14 hr^{-1}, but no significant trend with polyglutamate chain length could be ascertained. Efflux of higher MTX polyglutamates was shown to be significant over the 24-48 hr experimental period considered. MTX1, MTX2, and the MTX3 to MTX5 group had characteristic efflux times .17 hr, 3.2 hr, and 14.6 hr, comparing well with observations on MDA.MB.436 breast cancer cells. The model also was shown capable of predicting the fractions of the MTX polyglutamates bound at any time to reductase.

SIGNIFICANCE: These results show that several (3) human breast cancer lines exhibit similar qualitative polyglutamation behavior, and suggest that isolated folylpolyglutamyl synthase is a reasonable model of the enzyme in the intact cell. Most importantly, a model of MTX polyglutamation is now available for substitution into a general folate biochemistry model.

PROPOSED COURSE: Develop a kinetic model of MCF-7 folate biochemistry, and begin testing various MTX-induced purine inhibition hypotneses.

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